

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
4 March 2004 (04.03.2004)

PCT

(10) International Publication Number
WO 2004/018997 A2

(51) International Patent Classification⁷: **G01N**

(21) International Application Number:
PCT/US2003/026173

(22) International Filing Date: 20 August 2003 (20.08.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/405,417 20 August 2002 (20.08.2002) US
60/411,974 18 September 2002 (18.09.2002) US

(63) Related by continuation (CON) or continuation-in-part (CIP) to earlier applications:

US 60/405,417 (CIP)
Filed on 20 August 2002 (20.08.2002)
US 60/411,974 (CIP)
Filed on 18 September 2002 (18.09.2002)

(71) Applicant (for all designated States except US): **NEURO-GENETICS, INC.** [US/US]; 11085 North Torrey Pines Road, Suite 300, La Jolla, CA 92037 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **KOUNNAS, Maria** [US/US]; 3752 Vista de la Bahia, San Diego, CA 92117 (US). **PATRICK, Aaron** [US/US]; 1335 Monroe Street, #304, Denver, CO 80206 (US). **VELICELEBI, Gonul** [US/US]; 4688 Tarantella Lane, San Diego, CA 92130 (US). **WAGNER, Steven** [US/US]; 2315 Wilbur Avenue, San Diego, CA 92109 (US).

(74) Agents: **SEIDMAN, Stephanie, L.** et al.; Heller Ehrman White & McAuliffe LLP, 4350 La Jolla Village Drive, 7th Floor, San Diego, CA 92122-1246 (US).

(81) Designated States (national): AE, AG, AI., AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii)) for the following designations AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW, ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG)

— as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii)) for the following designations AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW, ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG)

Published:

— without international search report and to be republished upon receipt of that report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: METHODS AND COMPOSITIONS FOR MODULATING AMYLOID BETA

(57) Abstract: Provided herein are methods and compositions for detecting, assessing and modulating β -amyloid peptide (A β) levels and/or processing of amyloid precursor protein. Methods for screening and/or identifying agents that modulate processing of APP or the levels of β -amyloid peptides, and methods for assessing presenilin activity and for modulating lipoprotein receptor-related protein (LRP), are also provided.

METHODS AND COMPOSITIONS FOR MODULATING AMYLOID BETA

RELATED APPLICATIONS

Benefit of priority under §119(e) is claimed to U.S. Provisional Application
5 Serial No. 60/405,417, filed August 20, 2002, entitled "Methods of Modulating and
Identifying Agents that Modulate Processing of Amyloid Precursor Protein" and U.S.
Provisional Application Serial No. 60/411,974, filed September 18, 2002, entitled
"Methods of Modulating and Identifying Agents that Modulate Processing of Amyloid
Precursor Protein." The subject matter and contents, including sequence listings, of each
10 of these provisional applications is incorporated by reference herein in its entirety.

FIELD OF THE INVENTION

The field of invention relates to methods and compositions for detecting,
assessing and modulating β -amyloid peptide ($A\beta$) levels and processing of amyloid
precursor protein.

15 BACKGROUND

Alzheimer's disease (AD) is a progressive neurodegenerative disorder that is the
predominant cause of dementia in people over 65 years of age. It is estimated to affect 4
million Americans. Clinical symptoms of the disease begin with subtle short term
memory problems. As the disease progresses, difficulty with memory, language and
20 orientation worsen to the point of interfering with the ability of the person to function
independently. Other symptoms, which are variable, include myoclonus and seizures.
Duration of AD from the first symptoms of memory loss until death is 10 years on
average.

The AD brain is characterized by two distinct pathologies; 1) neurofibrillary
25 tangles (NFT), comprised mostly of tau and 2) amyloid plaques, comprised primarily of
highly hydrophobic amyloid precursor protein peptides called $A\beta$ peptides. The
characteristic Alzheimer's NFTs contain abnormal filaments bundled together in neurons
and occupying much of the perinuclear cytoplasm. These filaments contain the
microtubule-associated protein tau in a hyperphosphorylated form. "Ghost" NFTs are
30 also observed in AD brains, which presumably mark the location of dead neurons. $A\beta$

- 2 -

aggregates into antiparallel filaments in a β -pleated sheet structure resulting in the birefringent nature of the AD amyloid. Other neuropathological features include granulovascular changes, neuronal loss, gliosis and the variable presence of Lewy bodies.

Although $A\beta$ is the major component of AD amyloid, other proteins have also
 5 been found associated with amyloid plaques, *e.g.*, alpha-1-anti-chymotrypsin (Abraham
et al. (1988) *Cell* 52:487-501), cathepsin D (Cataldo (1990) *et al. Brain Res.* 513:181-
 192), non-amyloid component protein (Ueda *et al.* (1993) *Proc. Natl. Acad. Sci. U.S.A.*
 90:11282-11286), apolipoprotein E (apoE) (Namba *et al.* (1991) *Brain Res.* 541:163-
 166; Wisniewski and Frangione (1992) *Neurosci. Lett.* 135:235-238; Strittmatter *et al.*
 10 (1993) *Proc. Natl. Acad. Sci. U.S.A.* 90:1977-1981), apolipoprotein J (Choi-Mura (1992)
et al. Acta Neuropathol. 83:260-264; McGeer (1992) *et al. Brain Res.* 579:337-341), heat
 shock protein 70 (Hamos *et al.* (1991) *Neurology* 41:345-350), complement components
 (McGeer and Rogers (1992) *Neurology* 43:447-449), alpha2-macroglobulin (Strauss *et al.*
 (1992) *Lab. Invest.* 66:223-230), interleukin-6 (Strauss *et al.* (1992) *Lab. Invest.* 66:223-
 15 230), proteoglycans (Snow *et al.* (1987) *Lab. Invest.* 58:454-458), and serum amyloid P
 (Coria *et al.* (1988) *Lab. Invest.* 58:454-458).

Plaques are often surrounded by astrocytes and activated microglial cells
 expressing immune-related proteins, such as the MHC class II glycoproteins HLA-DR,
 HLA-DP and HLA-DQ, as well as MHC class I glycoproteins, interleukin-2 (IL-2)
 20 receptors and IL-1. Also surrounding many plaques are dystrophic neurites, which are
 nerve endings containing abnormal filamentous structures. Currently, there is no cure or
 effective treatment for AD and the few approved drugs including Aricept, Exelon,
 Cognex and Reminyl are palliative at best. Effective treatments are needed. Therefore,
 among the objects herein, it is an object to provide methods for modulating and for
 25 identifying agents for modulating the processing of amyloid precursor protein (APP) and
 the levels of $A\beta$ peptides. It is also an object to provide methods for identifying
 candidate agents for the treatment of AD and other neurodegenerative disorders
 characterized by altered levels of $A\beta$ peptides and/or amyloidosis.

- 3 -

SUMMARY

Provided herein are methods for assessing presenilin activity, comprising contacting a sample containing a presenilin and/or fragment(s) thereof with a lipoprotein receptor-related protein (LRP) and/or fragment(s) thereof; and assessing the processing and/or cleavage of the LRP or fragment(s) thereof. Also provided herein are methods for identifying an agent that modulates presenilin activity, comprising contacting a sample containing a presenilin, and/or fragment(s) thereof, and a lipoprotein receptor-related protein (LRP), and/or fragment(s) thereof with a test agent; and identifying an agent that alters the processing and/or cleavage of LRP and/or fragment(s) thereof. The processing and/or cleavage of LRP and/or fragment(s) thereof can be assessed by determining the presence, absence and/or level of one or more fragments of LRP and/or the composition of LRP. In one embodiment, the step of identifying comprises comparing the cleavage and/or processing of LRP and/or fragment(s) thereof in a test sample that has been contacted with the test agent and a control sample that has not been contacted with the test agent and identifying an agent as an agent that alters the processing and/or cleavage of LRP and/or fragment(s) thereof if the processing and/or cleavage of LRP and/or fragment(s) thereof differs in the test and control samples. The control sample can be the test sample in the absence of test agent. In certain embodiments, the processing or cleavage of LRP and/or fragment(s) thereof is assessed by determining the presence or absence and/or level of an LRP fragment that has a molecular weight of about 20 kD. The LRP fragment that has a molecular weight of about 20 kD can contain an amino acid sequence that is contained within a transmembrane region of LRP. In another embodiment, the LRP fragment that has a molecular weight of about 20 kD can bind with an antibody generated against a C-terminal amino acid sequence of an LRP. The C-terminal amino acid sequence of LRP can be a sequence of about the C-terminal 13 amino acids of an LRP. In another embodiment, the LRP fragment that has a molecular weight of about 20 kD comprises an amino acid sequence located within the sequence of amino acids from about amino acid 4420 to about amino acid 4544 of SEQ ID NO:10. The LRP fragment that has a molecular weight of about 20 kD can be present when an LRP is not cleaved by a presenilin-dependent activity; or can be in the presence of an

- 4 -

inhibitor of a presenilin-dependent activity. In a particular embodiment, the inhibitor is DAPT.

In particular embodiments of the methods provided herein, the processing and/or cleavage of LRP or fragment(s) thereof can be assessed by determining the presence or absence and/or level of an LRP C-terminal fragment (CTF). The processing and/or cleavage of LRP and/or fragment(s) thereof is assessed by determining the presence or absence and/or level of a fragment of LRP that binds to an antibody. The antibody can bind to an epitope in the C-terminal about 13 amino acids of an LRP, and can be a polyclonal antibody. The sample can comprise a composition selected from the group consisting of a cell, a tissue, an organism, a cell or tissue lysate, a cell or tissue extract, a body fluid, a cell membrane or composition containing cell membranes, and a cell-free extract or other cell-free sample. In particular embodiments, the cell can contain presenilin, LRP and/or fragment(s) of presenilin and/or LRP. The cell can be either eukaryotic, mammalian, rodent or a human cell.

Also provided herein are methods for identifying a candidate agent for treatment or prophylaxis of a disease associated with an altered presenilin, comprising contacting a sample that contains an altered presenilin and/or fragment(s) thereof and a lipoprotein receptor-related protein (LRP) and/or fragment(s) thereof with a test agent, wherein the altered presenilin and/or fragment(s) thereof is associated with an altered cleavage and/or processing of LRP and/or fragment(s) thereof; and identifying a candidate agent that restores LRP cleavage and/or processing to substantially that which occurs in the presence of a presenilin and/or fragment(s) thereof that is not associated with an altered cleavage and/or processing of LRP and/or fragment(s) thereof. The presenilin and/or fragment(s) thereof can comprise a mutation, and can be altered relative to a wild-type presenilin, wherein the wild-type is a predominant allele. The wild-type presenilin can be one that occurs in an organism that exhibits normal presenilin-dependent LRP processing patterns. The disease can be an amyloidosis-associated disease; a neurodegenerative disease, such as Alzheimer's Disease. The mutation can be linked to familial Alzheimer's disease.

In one embodiment of the methods provided herein, LRP cleavage and/or

- 5 -

processing is assessed by determining the presence or absence and/or level of one or more fragments of LRP and/or the composition of LRP. The step of identifying can comprise comparing the cleavage and/or processing of LRP and/or fragment(s) thereof in a test sample that has been contacted with the test agent and a control sample that has not
5 been contacted with the test agent and identifying an agent as a candidate agent that restores LRP cleavage and/or processing if the cleavage and/or processing of LRP and/or fragment(s) thereof differs in the test and control samples; or is substantially similar; wherein the positive control sample contains LRP and/or fragment(s) thereof and a presenilin and/or fragment(s) thereof that is not associated with an altered processing of
10 LRP. The presenilin and/or fragment(s) thereof in the positive control sample can be a wild-type presenilin. The cleavage or processing of LRP and/or fragment(s) thereof is assessed by determining the presence or absence and/or level of an LRP fragment that has a molecular weight of about 20 kD. The LRP fragment that has a molecular weight of about 20 kD can contain an amino acid sequence that is contained within a
15 transmembrane region of LRP; or can bind with an antibody generated against a C-terminal amino acid sequence of an LRP. The C-terminal amino acid sequence of LRP is a sequence of about the C-terminal 13 amino acids of an LRP. The LRP fragment that has a molecular weight of about 20 kD can comprise an amino acid sequence located within the sequence of amino acids from about amino acid 4420 to about amino acid
20 4544 of SEQ ID NO:10; can be one that is present when an LRP is not cleaved by a presenilin-dependent activity; or can occur in the presence of an inhibitor of a presenilin-dependent activity. In one embodiment, the inhibitor is DAPT. The LRP processing can be assessed by determining the presence or absence and/or level of an LRP C-terminal fragment (CTF); or by determining the presence or absence and/or level of a fragment of
25 LRP that binds to an antibody. The antibody can bind to an epitope in the C-terminal about 13 amino acids of an LRP, and can be a polyclonal antibody. The sample can comprise a composition selected from the group consisting of a cell, a tissue, an organism, a cell or tissue lysate, a cell or tissue extract, a body fluid, a cell membrane or composition containing cell membranes, and a cell-free extract or other cell-free sample.
30 In one embodiment, the sample can comprise a cell that contains the presenilin, LRP

- 6 -

and/or fragment(s) of presenilin and/or LRP. The cell can be eukaryotic, mammalian, rodent or a human cell.

Also provided herein are methods for modulating LRP, comprising altering the structure, function and/or activity of a presenilin, and/or fragment(s) thereof, in a sample comprising LRP, and/or fragment(s) thereof, and a presenilin, and/or fragment(s) thereof, whereby the LRP is modulated. In another embodiment, provided herein are methods for modulating LRP, comprising contacting a sample comprising an LRP, and/or fragment(s) thereof, and presenilin, and/or fragment(s) thereof, with an agent that modulates the presenilin and/or fragment(s) thereof or a presenilin-dependent activity, whereby LRP is modulated. In these methods the cleavage, processing, structure, function and/or activity of LRP can be modulated. The method can further comprise selecting a sample for modulation of LRP. The sample can comprise a composition selected from the group consisting of a cell, tissue, organism, cell or tissue lysate, cell or tissue extract, a cell membrane, a membrane preparation from a cell and a cell-free sample.

Also provide herein are methods for identifying an agent that modulates A β 42 levels, comprising comparing the levels of bound antibody and/or fragment(s) thereof in a test sample contacted with the test agent and a control sample not contacted with the test agent; and identifying an agent as an agent that modulates A β 42 levels if the levels of bound antibody differ in the test and control samples; wherein the sample comprises APP or portion(s) thereof; and the antibody and/or fragment(s) thereof comprises a sequence of amino acids selected from the group consisting of amino acids 1-50, 1-60, 1-70, 1-80, 1-90, 1-91, 1-92, 1-93, 1-94 and 1-95 of SEQ ID NO: 12 and any amino acid sequences containing modifications of these amino acid sequences that retain the antigen-binding properties of an antibody comprising one or both of the amino acid sequences set forth as amino acids 1-95 of SEQ ID NO: 12 and amino acids 1-97 of SEQ ID NO: 14. In another embodiment, provided herein are methods for identifying an agent that modulates A β 42 levels, comprising comparing the levels of bound antibody and/or fragment(s) thereof in a test sample contacted with the test agent and a control sample not contacted with the test agent; and identifying an agent as an agent that modulates A β 42 levels if the levels of bound antibody differ in the test and control samples;

- 7 -

wherein the sample comprises APP or portion(s) thereof; and the antibody and/or fragment(s) thereof comprises a sequence of amino acids selected from the group consisting of amino acids 1-50, 1-60, 1-70, 1-80, 1-90, 1-91, 1-92, 1-93, 1-94, 1-95, 1-96 and 1-97 of SEQ ID NO: 14 and any amino acid sequences containing modifications of these amino acid sequences that retain the antigen-binding properties of an antibody comprising one or both of the amino acid sequences set forth as amino acids 1-95 of SEQ ID NO: 12 and amino acids 1-97 of SEQ ID NO: 14. In these methods, the antibody comprising one or both of the amino acid sequences set forth as amino acids 1-95 of SEQ ID NO: 12 and amino acids 1-97 of SEQ ID NO: 14 can be an IgG. The antibody and/or fragment(s) thereof can bind A β 42 without substantially binding other A β forms, such as A β 40. The antibody and/or fragment(s) thereof can have at least about 100-fold, 200-fold, 300-fold, 400-fold, 500-fold, 600-fold, 700-fold, 800-fold, 900-fold, 1000-fold or more specificity or selectivity for A β 42 relative to other forms of A β , such as A β 40. In addition, the antibody and/or fragment(s) thereof can have an affinity constant for binding to A β 42 of at least about 10^5 l/mol, 2×10^5 l/mol, 3×10^5 l/mol, 4×10^5 l/mol, 5×10^5 l/mol, 6×10^5 l/mol, 7×10^5 l/mol, 8×10^5 l/mol, 9×10^5 l/mol, 10^6 l/mol, 2×10^6 l/mol, 3×10^6 l/mol or 4×10^6 l/mol or more. In one embodiment, the agent identified as an agent that modulates A β 42 levels can reduce A β 42 levels. The concentration of test agent can be less than or equal to about 35 μ M, 30 μ M, 25 μ M, 20 μ M, 15 μ M or 10 μ M. The step of identifying an agent as an agent that modulates A β 42 levels can comprise identifying an agent that reduces A β 42 levels with an IC_{50} of about 25 μ M or less or about 20 μ M or less.

Also provided herein are methods for identifying an agent that modulates A β levels, comprising assessing a test agent that modulates A β 42 levels to determine if it modulates the level of one or more other A β peptides; and identifying an agent that modulates A β 42 levels to a greater extent than it modulates the level of one or more other A β peptides. The step of identifying can comprise identifying an agent that modulates A β 42 levels without substantially altering the level of one or more other A β peptides, such as A β 40. The step of identifying can comprise identifying an agent that modulates A β 42 levels to a greater extent than it modulates the level of A β 40. In one

- 8 -

embodiment, the test agent reduces A β 42 levels. In another embodiment, the test agent increases A β 42 levels.

Further provided herein are methods for identifying an agent that modulates A β levels, comprising assessing a test agent that modulates A β 42 levels to determine if it modulates the level of one or more other A β peptides; and identifying an agent that modulates A β 42 levels and A β 39 levels. The test agent can reduce A β 42 levels or can increase A β 42 levels. The step of identifying can comprise identifying an agent that increases A β 39 or that reduces A β 39. In another embodiment, the step of identifying can comprise identifying an agent that modulates A β 42 levels and A β 39 levels to a greater extent than it modulates A β 40 levels. The step of identifying can comprise identifying an agent that modulates A β 42 levels and A β 39 without substantially altering the level of A β 40. In one embodiment, the step of assessing a test agent can comprise comparing the levels of one or more A β peptides other than A β 42 in a test sample that has been contacted with the test agent and in a control sample that has not been contacted with the test agent; and the step of identifying comprises identifying an agent as an agent that modulates A β 42 levels to a greater extent than it modulates the level of one or more other A β peptides if the difference in the levels of one or more A β peptides other than A β 42 in the test and control samples is less than the difference in the A β 42 levels of the test and control samples. In another embodiment, the step of assessing a test agent can comprise comparing the levels of one or more A β peptides other than A β 42 in a test sample that has been contacted with the test agent and in a control sample that has not been contacted with the test agent; and the step of identifying comprises identifying an agent as an agent that modulates A β 42 levels to a greater extent than it modulates the level of one or more other A β peptides if the levels of one or more A β peptides other than A β 42 in the test and control samples are substantially unchanged. In another embodiment, the step of assessing a test agent can comprise comparing the levels of A β 39 in a test sample that has been contacted with the test agent and in a control sample that has not been contacted with the test agent; and the step of identifying comprises identifying an agent as an agent that modulates A β 42 levels and A β 39 levels if A β 39 levels in the test and control samples differ.

- 9 -

Likewise, the step of identifying can comprise identifying an agent as an agent that modulates A β 42 levels and A β 39 levels to a greater extent than it modulates the level of A β 40 if the difference in the levels of A β 40 in a test sample that has been contacted with the test agent and a control sample that has not been contacted with a test agent is less than the difference in the A β 42 and A β 39 levels of the test and control samples. The step of identifying can also comprise identifying an agent as an agent that modulates A β 42 levels and A β 39 levels to a greater extent than it modulates the level of A β 40 if the levels of A β 40 in test sample that has been contacted with test agent and a control sample that has not been contacted with test agent are substantially unchanged.

The methods can further comprise a step of identifying the test agent as an agent that modulates A β 42 levels; wherein the step of identifying the test agent as an agent that modulates A β 42 levels is performed prior to or simultaneously with the step of assessing the test agent; and if the test agent is identified as an agent that modulates A β 42 levels simultaneously with the step of assessing the test agent, then the step of assessing includes determining if the test agent modulates the level of A β 42. The step of identifying the test agent as an agent that modulates A β 42 levels can comprise comparing the levels of A β 42 in a test sample that has been contacted with the test agent and in a control sample that has not been contacted with the test agent; and identifying a test agent as an agent that modulates A β 42 levels if the levels of A β 42 in the test and control samples differ. The levels of A β 42 in the samples are assessed in a method comprising an immunoassay wherein an antibody and/or fragment(s) thereof that bind A β 42 without substantially binding other A β forms is used. The antibody and/or fragment(s) thereof can be at least about 100-fold, 200-fold, 300-fold, 400-fold, 500-fold, 600-fold, 700-fold, 800-fold, 900-fold, 1000-fold or more specificity or selectivity for A β 42 relative to other forms of A β , such as A β 40. The antibody and/or fragment(s) thereof bind A β 42 without substantially binding A β 40. In one embodiment, the test agent can reduce A β 42 levels. In the step of identifying the test agent as an agent that modulates A β 42 levels, the concentration of test agent can be less than or equal to about 35 μ M, 30 μ M, 25 μ M, 20 μ M, 15 μ M or 10 μ M. The step of identifying the test agent as an agent that modulates A β 42 levels can comprise identifying an agent that reduces A β 42 levels with an IC₅₀ of

- 10 -

about 25 μ M or less or about 20 μ M or less. The sample can comprise APP and/or portion(s) thereof. In other embodiments, the sample can comprise a composition selected from the group consisting of a cell, a tissue, an organism, a cell or tissue lysate, a cell or tissue extract, a body fluid, a cell membrane or composition containing cell
5 membranes, and a cell-free extract or other cell-free sample. The sample can comprise a cell. The A β can be a cellular and/or extracellular A β .

Also provided herein are methods for modulating A β levels of a sample, comprising altering the A β peptide-producing cleavage of APP, the processing of APP, the processing of A β and/or the levels of A β such that the level of A β 42 is modulated to
10 a greater extent than the level of one or more other A β peptides is modulated. Also provided are methods for modulating A β levels of a sample, comprising contacting a sample comprising APP and/or a portion(s) thereof with an agent that modulates the level of A β 42 to a greater extent than the level of one or more other A β peptides. The level of A β 42 can be modulated without substantially altering the level of one or more other A β
15 peptides. The level of A β 42 can be modulated to a greater extent than the level of A β 40. The level of A β 42 is modulated without substantially altering the level of A β 40. The level of A β 42 can be reduced or increased. The level of A β 42 and the level of A β 39 can be modulated to a greater extent than the level of one or more other A β peptides, such as A β 40. The level of A β 42 and the level of A β 39 can be modulated without substantially
20 altering the level of one or more other A β peptides, such as A β 40. In particular embodiments, the level of A β 42 is reduced; the level of A β 39 is increased; or the level of A β 42 is increased. The concentration of the agent can be less than or equal to about 35 μ M, 30 μ M, 25 μ M, 20 μ M, 15 μ M or 10 μ M. The sample can comprise APP and/or portion(s) thereof. The A β can be a cellular and/or extracellular A β .

25 Also provided herein are methods for identifying an agent that modulates A β levels, comprising assessing a test agent that alters the cleavage of APP that produces one or more A β peptides, the processing of APP, the processing of A β and/or the level of one or more A β peptides to determine if it effects one or more presenilin-dependent activities other than the presenilin-dependent processing of APP or portion(s) thereof;
30 and identifying an agent that modulates A β levels without substantially altering one or

- 11 -

more presenilin-dependent activities other than the presenilin-dependent processing of APP.

In the methods provided herein, the test agent can modulate A β 42 levels, such as to a greater extent than it modulates the levels of other A β peptides; without substantially altering the level of one or more other A β peptides; to a greater extent than it modulates the levels of A β 40; or without substantially altering the level of A β 40. In another embodiment, the test agent can modulate A β 42 and A β 39 levels, such as to a greater extent than it modulates the levels of other A β peptides; without substantially altering the level of one or more other A β peptides; to a greater extent than it modulates the levels of A β 40; or without substantially altering the level of A β 40. In these methods, the step of assessing a test agent can comprise comparing one or more presenilin-dependent activities other than the presenilin-dependent processing of APP and/or portion(s) thereof in a test sample that has been contacted with the test agent and in a control sample that has not been contacted with the test agent; and the step of identifying comprises identifying an agent that modulates A β levels without substantially altering one or more presenilin-dependent activities other than the presenilin-dependent processing of APP if the one or more presenilin-dependent activities other than the presenilin-dependent processing of APP is (are) substantially unchanged in the test and control samples. The presenilin-dependent activity other than presenilin-dependent processing of APP can be the cleavage and/or processing of a substrate, and/or portion(s) thereof, other than APP. The test agent can reduce or increase A β 42 levels.

Also provided herein are methods for identifying an agent that modulates A β levels, comprising assessing a test agent that modulates the cleavage of APP that produces one or more A β peptides, the processing of APP, the processing of A β and/or the level of one or more A β peptides to determine if it effects the cleavage and/or processing of a presenilin substrate and/or portion(s) thereof other than APP or other than the presenilin-dependent processing of APP or portion(s) thereof; and identifying an agent that modulates A β levels without substantially altering the cleavage and/or processing of the presenilin substrate and/or portion(s) thereof that is other than APP. The step of assessing the test agent can comprise comparing (a) the cleavage and/or

- 12 -

processing of a presenilin substrate, and/or portion(s) thereof, other than APP, and/or (b) the levels of a fragment(s) of the presenilin substrate and/or portion(s) thereof in a test sample that has been contacted with the test agent and in a control sample that has not been contacted with the test agent; and the step of identifying comprises identifying an agent if the cleavage and/or processing of the presenilin substrate and/or portion(s) thereof and/or the levels of a fragment(s) of the presenilin substrate and/or portion(s) thereof in the test and control samples do not substantially differ. The step of assessing can comprise comparing (a) the cleavage and/or processing of a presenilin substrate, and/or portion(s) thereof, other than APP and/or (b) the levels of a fragment(s) of the presenilin substrate and/or portion(s) thereof in a test sample that has been contacted with the test agent and a positive control sample; and the step of identifying comprises identifying an agent if the cleavage and/or processing of a presenilin substrate, and/or portion(s) thereof, and/or the levels of fragment(s) of the presenilin substrate and/or portion(s) thereof in the test and positive control samples substantially differ; wherein the positive control sample is one that has been contacted with an modulator of presenilin and/or presenilin-dependent activity. The modulator of presenilin and/or presenilin-dependent activity is an inhibitor of presenilin and/or presenilin-dependent activity. The inhibitor can be DAPT. The level of a substrate fragment in the test sample can be less than about 40%, 35%, 30% or 20% of the level of the fragment in the positive control sample. The concentration of test agent is less than or equal to about 35 μ M, 30 μ M, 25 μ M, 20 μ M, 15 μ M or 10 μ M. The sample comprises a presenilin substrate and/or portion(s) thereof; and/or presenilin and/or portion(s) thereof. The method can further comprise a step of identifying the test agent as an agent that modulates A β levels; wherein the step of identifying the test agent as an agent that modulates A β levels is performed prior to or simultaneously with the step of assessing the test agent; and if the test agent is identified as an agent that modulates A β levels simultaneously with the step of assessing the test agent, then the step of assessing includes determining if the test agent modulates A β levels. The step of identifying the test agent as an agent that modulates the cleavage of APP and/or portion(s) thereof that produces one or more A β peptides, the processing of APP, the processing of A β and/or the level of one or more A β

- 13 -

peptides can comprise comparing the A β peptide-producing cleavage of APP, or portion(s) thereof, APP processing, A β processing and/or A β levels in a test sample containing APP and/or portion(s) thereof that has been contacted with the test agent and in a control sample that has not been contacted with the test agent; and identifying an agent as an agent that modulates A β levels if the A β peptide-producing cleavage of APP, or portion(s) thereof, APP processing, A β processing and/or A β levels in the test and control samples differ. The presenilin substrate and/or portion(s) thereof can be selected from the group consisting of LRP, Notch, E-cadherin, Erb-B4, and portions of LRP, Notch, E-cadherin and Erb-B4. The step of assessing the test agent can comprise comparing the levels of an intracellular carboxyl domain fragment of Notch, E-cadherin and/or Erb-B4 in test and control samples; and the step of identifying comprises identifying an agent if the cleavage and/or processing of Notch, E-cadherin and/or Erb-B4 (and/or portion(s) thereof) and/or the levels of an intracellular carboxyl domain fragment of Notch, E-cadherin and/or Erb-B4 in the test and control samples do not substantially differ. The sample can comprise a composition selected from the group consisting of a cell, a tissue, an organism, a cell or tissue lysate, a cell or tissue extract, a body fluid, a cell membrane or composition containing cell membranes, and a cell-free extract or other cell-free sample.

Also provided herein are methods for identifying an agent that modulates A β levels, comprising assessing a test agent that modulates the cleavage of APP that produces one or more A β peptides, the processing of APP, the processing of A β and/or the level of one or more A β peptides to determine if it effects the cleavage and/or processing of LRP and/or portion(s) thereof; and identifying an agent that modulates A β levels without substantially altering the cleavage and/or processing of LRP and/or portion(s) thereof. The test agent can modulate A β 42 levels; modulate A β 42 levels to a greater extent than it modulates the levels of other A β peptides; modulate A β 42 levels without substantially altering the level of one or more other A β peptides; modulate A β 42 levels to a greater extent than it modulates the levels of A β 40; modulate A β 42 levels without substantially altering the level of A β 40; modulate A β 42 and A β 39 levels; modulate A β 42 and A β 39 levels to a greater extent than it modulates the levels of other

- 14 -

A β peptides; modulate A β 42 and A β 39 levels without substantially altering the level of one or more other A β peptides; modulate A β 42 and A β 39 levels to a greater extent than it modulates the levels of A β 40; modulate A β 42 and A β 39 levels without substantially altering the level of A β 40; reduces A β 42 levels; increases A β 39 levels. The step of

5 assessing the test agent can comprise comparing (a) the cleavage and/or processing of LRP, and/or portion(s) thereof, and/or (b) the levels of a fragment(s) of LRP and/or portion(s) thereof in a test sample that has been contacted with the test agent and in a control sample that has not been contacted with the test agent; and the step of identifying comprises identifying an agent if the cleavage and/or processing of LRP and/or portion(s)

10 thereof and/or the levels of a fragment(s) of LRP and/or portion(s) thereof in the test and control samples do not substantially differ. The step of assessing can comprise comparing (a) the cleavage and/or processing of LRP, and/or portion(s) thereof, and/or (b) the levels of a fragment(s) of LRP and/or portion(s) thereof in a test sample that has been contacted with the test agent and a positive control sample; and the step of

15 identifying comprises identifying an agent if the cleavage and/or processing of LRP, and/or portion(s) thereof, and/or the levels of fragment(s) of LRP and/or portion(s) thereof in the test and positive control samples substantially differ; wherein the positive control sample is one that has been contacted with an modulator of presenilin and/or presenilin-dependent activity. In one embodiment, the modulator of presenilin and/or

20 presenilin-dependent activity can be an inhibitor of presenilin and/or presenilin-dependent activity. The level of an LRP fragment in the test sample can be less than about 40%, 35%, 30% or 20% of the level of the fragment in the positive control sample. The concentration of test agent can be less than or equal to about 35 μ M, 30 μ M, 25 μ M, 20 μ M, 15 μ M or 10 μ M. The cleavage and/or processing of LRP, and/or portion(s)

25 thereof, and/or the levels of a fragment(s) of LRP and/or portion(s) thereof can be assessed by determining the presence, absence and/or level of one or more fragments of LRP and/or the composition of LRP. The cleavage and/or processing of LRP, and/or portion(s) thereof, and/or the levels of a fragment(s) of LRP and/or portion(s) thereof can be assessed by determining the presence, absence and/or level of an LRP fragment that

30 has a molecular weight of about 20 kD. The LRP fragment that has a molecular weight

- 15 -

of about 20 kD can contain an amino acid sequence that is contained within a transmembrane region of LRP; can bind with an antibody generated against a C-terminal amino acid sequence of an LRP, wherein the C-terminal amino acid sequence of LRP is a sequence of about the C-terminal 13 amino acids of an LRP; can comprise an amino acid
5 sequence located within the sequence of amino acids from about amino acid 4420 to about amino acid 4544 of SEQ ID NO:10; is one that is present when an LRP is not cleaved by a presenilin-dependent activity; is one that occurs in the presence of an inhibitor of a presenilin-dependent activity. The cleavage and/or processing of LRP, and/or portion(s) thereof, and/or the levels of a fragment(s) of LRP and/or portion(s)
10 thereof can be assessed by determining the presence, absence and/or level of an LRP C-terminal fragment (CTF); can be assessed by determining the presence or absence and/or level of a fragment of LRP that binds to an antibody. The sample can comprise LRP and/or portion(s) thereof; or presenilin and/or portion(s) thereof. The method can further comprise a step of identifying the test agent as an agent that modulates A β levels;
15 wherein the step of identifying the test agent as an agent that modulates A β levels is performed prior to or simultaneously with the step of assessing the test agent; and if the test agent is identified as an agent that modulates A β levels simultaneously with the step of assessing the test agent, then the step of assessing includes determining if the test agent modulates A β levels. The step of identifying the test agent as an agent that
20 modulates the cleavage of APP and/or portion(s) thereof that produces one or more A β peptides, the processing of APP, the processing of A β and/or the level of one or more A β peptides can further comprise: comparing the A β peptide-producing cleavage of APP, or portion(s) thereof, APP processing, A β processing and/or A β levels in a test sample containing APP and/or portion(s) thereof that has been contacted with the test agent and
25 in a control sample that has not been contacted with the test agent; and identifying an agent as an agent that modulates A β levels if the A β peptide-producing cleavage of APP, or portion(s) thereof, APP processing, A β processing and/or A β levels in the test and control samples differ. The sample comprises a composition selected from the group consisting of a cell, a tissue, an organism, a cell or tissue lysate, a cell or tissue extract, a
30 body fluid, a cell membrane or composition containing cell membranes, and a cell-free

- 16 -

extract or other cell-free sample. The step of identifying the test agent as an agent that modulates A β levels can comprise identifying an agent that reduces A β 42 levels in test samples contacted with the test agent by greater than or equal to about 50% compared to the levels of A β 42 in a control sample that has not been contacted with the agent. The
5 concentration of the identified agent can be less than or equal to about 35 μ M, 30 μ M, 25 μ M, 20 μ M, 15 μ M or 10 μ M. In one embodiment, the step of identifying an agent that modulates A β levels without substantially altering the cleavage and/or processing of LRP and/or portion(s) thereof can comprise identifying an agent if the level of an ~20 kD
10 positive control sample that has been contacted with an inhibitor of presenilin and/or presenilin-dependent activity. In one embodiment, the A β levels are extracellular levels and the LRP fragment levels are cellular levels.

Further provided herein are methods of modulating the A β levels of a sample, comprising modulating the cleavage of APP that produces one or more A β peptides, the
15 processing of APP, the processing of A β and/or the level of one or more A β peptides without substantially altering one or more presenilin-dependent activities other than the presenilin-dependent processing of APP. In one embodiment, the modulating can be effected by contacting the sample with an agent that modulates the cleavage of APP that produces one or more A β peptides, the processing of APP, the processing of A β and/or
20 the level of one or more A β peptides without substantially altering one or more presenilin-dependent activities other than the presenilin-dependent processing of APP. Also provided are methods of modulating the A β levels of a sample, comprising modulating the cleavage of APP that produces one or more A β peptides, the processing of APP, the processing of A β and/or the level of one or more A β peptides without
25 substantially altering the cleavage and/or processing of a presenilin substrate and/or portion(s) thereof that is other than APP. In one embodiment, the modulating can be effected by contacting the sample with an agent that modulates the cleavage of APP that produces one or more A β peptides, the processing of APP, the processing of A β and/or the level of one or more A β peptides without substantially altering the cleavage and/or
30 processing of a presenilin substrate and/or portion(s) thereof that is other than APP.

- 17 -

Further provided are methods of modulating the A β levels of a sample, comprising modulating the cleavage of APP that produces one or more A β peptides, the processing of APP, the processing of A β and/or the level of one or more A β peptides without substantially altering the cleavage and/or processing of LRP and/or portion(s) thereof,

5 wherein modulating can be effected by contacting the sample with an agent that modulates the cleavage of APP that produces one or more A β peptides, the processing of APP, the processing of A β and/or the level of one or more A β peptides without substantially altering the cleavage and/or processing of LRP and/or portion(s) thereof. In the above methods, the levels of A β 42 can be modulated: to a greater extent than the

10 levels of other A β peptides; without substantially altering the level of one or more other A β peptides; to a greater extent than the levels of A β 40; without substantially altering the level of A β 40; and the like. In other embodiments, the levels of A β 42 and A β 39 can be modulated, such as: to a greater extent than the levels of other A β peptides; without substantially altering the level of one or more other A β peptides; to a greater extent than

15 the levels of A β 40; without substantially altering the level of A β 40. In other embodiments, the level of A β 42 can be reduced or increased. Likewise, the level of A β 39 can be increased or reduced. The sample can comprise presenilin and/or portion(s) thereof; APP and/or portion(s) thereof; a presenilin substrate and/or portion(s) thereof; and the like. The sample can comprise one or more of LRP, Notch, E-cadherin, TrkB,

20 APLP2, hIre1 α , Erb-B4, portion(s) of LRP, portion(s) of Notch, portion(s) of E-cadherin, portion(s) of TrkB, portion(s) of APLP2, portion(s) of hIre1 α , and portion(s) of Erb-B4. The sample can comprise a composition selected from the group consisting of a cell, a tissue, an organism, a cell or tissue lysate, a cell or tissue extract, a body fluid, a cell membrane or composition containing cell membranes, and a cell-free extract or other

25 cell-free sample. In one embodiment, the sample comprises a cell, such as a eukaryotic, a mammalian, a rodent or a human cell. The A β can be a cellular and/or extracellular A β . In particular embodiments of these methods, the A β 42 levels of the sample can be reduced by greater than or equal to about 50%. The presenilin substrate and/or portion(s) thereof can be selected from the group consisting of Notch, E-cadherin, Erb-B4, and

30 portions of Notch, E-cadherin and Erb-B4. In one embodiment, the levels of an

- 18 -

intracellular carboxyl domain fragment of Notch, E-cadherin and/or Erb-B4 are substantially unchanged. In another, the level or absence of an ~20 kD fragment of LRP is substantially unchanged. In other embodiments, the LRP fragment that has a molecular weight of about 20 kD: can contain an amino acid sequence that is contained
5 within a transmembrane region of LRP; or can bind with an antibody generated against a C-terminal amino acid sequence of an LRP, wherein the C-terminal amino acid sequence of LRP is a sequence of about the C-terminal 13 amino acids of an LRP. In other embodiments, the LRP fragment that has a molecular weight of about 20 kD: can comprise an amino acid sequence located within the sequence of amino acids from about
10 amino acid 4420 to about amino acid 4544 of SEQ ID NO:10; can be one that is present when an LRP is not cleaved by a presenilin-dependent activity; can be one that occurs in the presence of an inhibitor of a presenilin-dependent activity. The inhibitor can be DAPT. In these methods, the level or absence of an LRP-CTF can be substantially unchanged. The concentration of agent can be less than or equal to about 35 μ M, 30 μ M,
15 25 μ M, 20 μ M, 15 μ M or 10 μ M. In a particular embodiment, the agent reduces A β 42 levels with an IC₅₀ of about 25 μ M or less or about 20 μ M or less.

Also provided herein are methods for treating or preventing a disease or disorder, comprising administering to a subject an agent that modulates the A β peptide-producing cleavage of APP, the processing of APP, the processing of A β and/or the levels of A β
20 such that the level of A β 42 is modulated to a greater extent than the level of one or more other A β peptides is modulated. The level of A β 42 can be modulated: without substantially altering the level of one or more other A β peptides; to a greater extent than the level of A β 40; without substantially altering the level of A β 40. In one embodiment, the level of A β 42 is reduced. In other embodiments, the level of A β 42 and the level of
25 A β 39 can be modulated to a greater extent than the level of one or more other A β peptides; to a greater extent than the level of A β 40; without substantially altering the level of one or more other A β peptides; without substantially altering the level of A β 40. The level of A β 42 can be reduced or increased.

Also provided herein are methods for treating or preventing a disease or disorder,
30 comprising administering to a subject an agent that modulates the A β peptide-producing

- 19 -

cleavage of APP, the processing of APP, the processing of A β and/or the levels of A β without substantially altering one or more presenilin-dependent activities other than the presenilin-dependent processing of APP. Also provided are methods for treating or preventing a disease or disorder, comprising administering to a subject an agent that
5 modulates the A β peptide-producing cleavage of APP, the processing of APP, the processing of A β and/or the levels of A β without substantially altering the cleavage and/or processing of a presenilin substrate and/or portion(s) thereof that is other than APP.

Also provided herein are methods for treating or preventing a disease or disorder,
10 comprising administering to a subject an agent that modulates the A β peptide-producing cleavage of APP, the processing of APP, the processing of A β and/or the levels of A β without substantially altering the cleavage and/or processing of LRP and/or portion(s) thereof. The level of A β 42 can be modulated: to a greater extent than the levels of other A β peptides; to a greater extent than the levels of other A β peptides; without
15 substantially altering the level of one or more other A β peptides; to a greater extent than the level of A β 40; without substantially altering the level of A β 40. In one embodiment, the level of A β 42 is reduced. In other embodiments, the level of A β 42 and the level of A β 39 can be modulated: to a greater extent than the level of one or more other A β peptides; to a greater extent than the level of A β 40; without substantially altering the
20 level of one or more other A β peptides; without substantially altering the level of A β 40. The level of A β 42 can be reduced or increased. The presenilin substrate and/or portion(s) thereof can be selected from the group consisting of Notch, E-cadherin, Erb-B4, and portions of Notch, E-cadherin and Erb-B4.

With respect to any of the methods provided herein for treating a disease or
25 disorder, the disease or disorder can be one characterized by altered A β production, catabolism, processing and/or levels. The disease or disorder can be one associated with amyloidosis, can be a neurodegenerative disease, and in a particular embodiment, is Alzheimer's disease.

Also provided are systems for use in assessing presenilin activity, comprising a
30 source of presenilin activity; a source of LRP protein; and a reagent for determining LRP

- 20 -

protein composition. The reagent for determining LRP protein composition: can bind to LRP protein or a fragment of an LRP protein; can be an antibody or portion of an antibody that binds to LRP; can bind to a C-terminal portion of LRP; can bind to an ~20 kD fragment of LRP. The LRP fragment that has a molecular weight of about 20 kD; can contain an amino acid sequence that is contained within a transmembrane region of LRP; can bind with an antibody generated against a C-terminal amino acid sequence of an LRP. The C-terminal amino acid sequence of LRP can be a sequence of about the C-terminal 13 amino acids of an LRP. In other embodiments, the LRP fragment that has a molecular weight of about 20 kD: can comprise an amino acid sequence located within the sequence of amino acids from about amino acid 4420 to about amino acid 4544 of SEQ ID NO:10; can be one that is present when an LRP is not cleaved by a presenilin-dependent activity; can be one that occurs in the presence of an inhibitor of a presenilin-dependent activity. The inhibitor can be DAPT. The source of a presenilin activity can be selected from the group consisting of a cell comprising a presenilin, an extract of a cell comprising a presenilin and medium comprising a presenilin.

Also provided herein are antibodies or fragments thereof comprising the sequence of amino acids 1-95 as set forth in SEQ ID NO: 12 and/or the sequence of amino acids 1-97 as set forth in SEQ ID NO: 14. The antibody or fragment thereof can comprise a light chain variable region that comprises the sequence of amino acids 1-95 as set forth in SEQ ID NO: 12. In another embodiment, the antibody or fragment thereof can comprise a light chain variable region that comprises the sequence of amino acids selected from the group consisting of 1-50, 1-60, 1-70, 1-80, 1-90, 1-91, 1-92, 1-93, and 1-94 of SEQ ID NO: 12. The antibody or fragment thereof can comprise a heavy chain variable region that comprises the sequence of amino acids 1-97 as set forth in SEQ ID NO: 14. The antibody or fragment thereof can comprise a heavy chain variable region that comprises the sequence of amino acids selected from the group consisting of 1-50, 1-60, 1-70, 1-80, 1-90, 1-91, 1-92, 1-93, 1-94, 1-95, and 1-96 of SEQ ID NO: 14. The antibodies or fragments thereof can further comprise one or more joining regions. In one embodiment, at least one joining region comprises the sequence of amino acids 96-107 as set forth in SEQ ID NO: 12. The antibody or fragment thereof can further comprise one or more

- 21 -

constant regions, wherein at least one constant region is a mouse constant region. The mouse constant region can comprise the sequence of amino acids selected from the group consisting of SEQ ID NOs: 63 and 65. In another embodiment, the at least one constant region is a human constant region.

- 5 The human constant region can comprise the sequence of amino acids as set forth in SEQ ID NO: 81. The at least one joining region can comprise the sequence of amino acids 98-118 as set forth in SEQ ID NO: 14. In this embodiment, the antibody or fragment thereof can further comprise one or more constant regions. The at least one constant region can be a mouse constant region. The mouse constant region can
- 10 comprise the sequence of amino acids selected from the group consisting of SEQ ID NOs: 69 and 71. The at least one constant region can be a human constant region. The human constant region can comprise the sequence of amino acids selected from the group consisting of SEQ ID NOs: 83, 85 and 87. The at least one joining region can comprise a mouse joining region. The mouse joining region can comprise the sequence
- 15 of amino acids selected from the group consisting of SEQ ID NOs: 46, 48, 50, 52, 54, 55, 57, 59, 61 and 67. The antibody or fragment thereof can further comprise one or more constant regions, wherein at least one constant region is a mouse constant region. The mouse constant region can comprise the sequence of amino acids selected from the group consisting of SEQ ID NOs: 63, 65, 69 and 71. The at least one constant region can be a
- 20 human constant region. The human constant region can comprise the sequence of amino acids selected from the group consisting of SEQ ID NOs: 81, 83, 85 and 87. In another embodiment, the at least one joining region can comprise a human joining region. The human joining region can comprise the sequence of amino acids selected from the group consisting of SEQ ID NOs: 73, 75, 77, 79, 89 and 91. The antibody or fragment thereof
- 25 can further comprise one or more constant regions, wherein at least one constant region is a mouse constant region. The mouse constant region can comprise the sequence of amino acids selected from the group consisting of SEQ ID NOs: 63, 65, 69 and 71. The at least one constant region can be a human constant region. The human constant region can comprise the sequence of amino acids selected from the group consisting of SEQ ID
- 30 NOs: 81, 83, 85 and 87.

- 22 -

Also provide herein is an antibody or fragment thereof encoded by the sequence of nucleic acids as set forth in SEQ ID NO: 11 and/or the sequence of nucleic acids as set forth in SEQ ID NO: 13. The antibody or fragment thereof can comprise: a light chain variable region encoded by the sequence of nucleic acids as set forth in SEQ ID NO: 11; or a heavy chain variable region encoded by the sequence of nucleic acids as set forth in SEQ ID NO: 13.

Also provided herein is an antibody or fragment thereof comprising the sequence of amino acids as set forth in SEQ ID NO: 97 and/or the sequence of amino acids as set forth in SEQ ID NO: 98. In embodiment, the antibody reacts with A β 42 with an affinity of at least about 4×10^6 l/mol. In another embodiment, the antibody reacts with A β 42 with an affinity of at least about 10^8 l/mol, or 10^9 l/mol or 10^{10} l/mol. The antibody or fragment thereof can comprise at least a portion of the antigen-binding region of the antibody, wherein the portion binds to the same antigenic determinant as the antibody with an affinity of at least about 1%, 5%, 10%, 50%, 70%, 80% or 100% of the entire antibody. Further provided is an antibody or fragment thereof comprising the sequence of amino acids 1-100 as set forth in SEQ ID NO: 16 and/or the sequence of amino acids 1-98 as set forth in SEQ ID NO: 18. The antibody or fragment thereof can comprise a light chain variable region that comprises the sequence of amino acids 1-100 as set forth in SEQ ID NO: 16. The antibody or fragment thereof can comprise a light chain variable region that comprises the sequence of amino acids selected from the group consisting of 1-50, 1-60, 1-70, 1-80, 1-90, 1-95, 1-96, 1-97, 1-98, and 1-99 of SEQ ID NO: 16. The antibody or fragment thereof can comprise a heavy chain variable region that comprises the sequence of amino acids 1-98 as set forth in SEQ ID NO: 18. The antibody or fragment thereof can comprise a heavy chain variable region that comprises the sequence of amino acids selected from the group consisting of 1-50, 1-60, 1-70, 1-80, 1-90, 1-91, 1-92, 1-93, and 1-94, 1-95, 1-96, and 1-97 of SEQ ID NO: 18. The antibody or fragment thereof can further comprise one or more joining regions, wherein at least one joining region comprises the sequence of amino acids 101-112 as set forth in SEQ ID NO: 16. The antibody or fragment thereof can further comprise one or more constant regions. At least one constant region can be a mouse constant region. The mouse

- 23 -

constant region can comprise the sequence of amino acids selected from the group consisting of SEQ ID NOs: 63 and 65. In another embodiment, at least one constant region is a human constant region. The human constant region can comprise the sequence of amino acids as set forth in SEQ ID NO: 81. In another embodiment, at least one joining region can comprise the sequence of amino acids 99-114 as set forth in SEQ ID NO: 14. The antibody or fragment thereof can further comprise one or more constant regions, wherein at least one constant region is a mouse constant region. The mouse constant region can comprise the sequence of amino acids selected from the group consisting of SEQ ID NOs: 69 and 71. In another embodiment, at least one constant region can be a human constant region. The human constant region can comprise the sequence of amino acids selected from the group consisting of SEQ ID NOs: 83, 85 and 87. In one embodiment, at least one joining region comprises a mouse joining region. The mouse joining region can comprise the sequence of amino acids selected from the group consisting of SEQ ID NOs: 46, 48, 50, 52, 54, 55, 57, 59, 61 and 67. The antibody or fragment thereof can further comprise one or more constant regions. In another embodiment, at least one constant region can be a mouse constant region. The mouse constant region can comprise the sequence of amino acids selected from the group consisting of SEQ ID NOs: 63, 65, 69 and 71. In another embodiment, at least one constant region is a human constant region. The human constant region can comprise the sequence of amino acids selected from the group consisting of SEQ ID NOs: 81, 83, 85 and 87. At least one joining region can comprise a human joining region. The human joining region can comprise the sequence of amino acids selected from the group consisting of SEQ ID NOs: 73, 75, 77, 79, 89 and 91. The antibody or fragment thereof can further comprise one or more constant regions, wherein at least one constant region is a mouse constant region or a human constant region. The mouse constant region can comprise the sequence of amino acids selected from the group consisting of SEQ ID NOs: 63, 65, 69 and 71. The human constant region can comprise the sequence of amino acids selected from the group consisting of SEQ ID NOs: 81, 83, 85 and 87.

Also provided herein is an antibody or fragment thereof encoded by the sequence of nucleic acids as set forth in SEQ ID NO: 15 and/or the sequence of nucleic acids as set

- 24 -

forth in SEQ ID NO: 17. The antibody or fragment thereof can comprise a light chain variable region encoded by the sequence of nucleic acids as set forth in SEQ ID NO: 15 and/or a heavy chain variable region encoded by the sequence of nucleic acids as set forth in SEQ ID NO: 17. Also provided is an antibody or fragment thereof comprising the
5 sequence of amino acids as set forth in SEQ ID NO: 99 and/or the sequence of amino acids as set forth in SEQ ID NO: 100.

Also provided herein is a protein or fragment thereof comprising the sequence of amino acids 1-95 as set forth in SEQ ID NO: 12 and/or the sequence of amino acids 1-97 as set forth in SEQ ID NO: 14. Further provided herein is a protein or fragment thereof
10 comprising the sequence of amino acids as set forth in SEQ ID NO: 97 and/or the sequence of amino acids as set forth in SEQ ID NO: 98. Also provided is a protein or fragment thereof comprising the sequence of amino acids 1-100 as set forth in SEQ ID NO: 16 and/or the sequence of amino acids 1-98 as set forth in SEQ ID NO: 18. Further
15 provided is a protein or fragment thereof comprising the sequence of amino acids as set forth in SEQ ID NO: 99 and/or the sequence of amino acids as set forth in SEQ ID NO: 100. Also provided herein is an isolated nucleic acid molecule that encoding these proteins. Also provided are isolated nucleic acid molecules that encode the antibodies provided herein.

Also provided herein are assays for determining the A β 42 content of a sample,
20 comprising contacting an antibody or fragment thereof provided herein with the sample under conditions whereby the antibody forms complexes with A β ; and determining if the antibody or fragment thereof binds to a molecule in the sample. The A β can be A β 42. The assay can be an enzyme-linked immunosorbant assay (ELISA). The antibody can be a capture antibody. The binding of the antibody or fragment thereof to a molecule in the
25 sample can be determined by contacting the complex with a second antibody or fragment thereof, such as, for example an antibody or fragment thereof provided herein that contains the sequence of amino acids 1-100 of SEQ ID NO: 16 and/or the sequence of amino acids 1-98 of SEQ ID NO: 18.

Also provided herein is a kit containing a reagent for assessing cleavage of APP
30 that produces one or more A β peptides, APP processing, A β processing and/or A β levels

- 25 -

and a reagent for assessing cleavage and/or processing of a presenilin substrate. In one embodiment, the presenilin substrate is LRP and/or portion(s) thereof. The reagent for assessing A β levels can be, for example, an antibody and/or fragment(s) thereof that specifically react with A β 42, such as any of the A β 42 specific antibodies provided

5 herein. A reagent for assessing A β levels can include an antibody and/or fragment(s) thereof that reacts with two or more or most A β peptides, such as antibodies provided herein that contains the sequence of amino acids 1-100 of SEQ ID NO: 16 and/or the sequence of amino acids 1-98 of SEQ ID NO: 18. The reagent for assessing cleavage and/or processing of LRP can be an antibody and/or fragment(s) thereof that recognizes a

10 fragment of LRP. The antibody can be one that prepared against the carboxyl-terminal 13 amino acid peptide of LRP (C-GRGPEDEIGDPLA). The LRP fragment can be one that is generated by a presenilin-dependent activity or a fragment that occurs in the absence of such activity. The fragment can have a molecular weight of about 20 kD.

Also provided is a method for identifying a candidate agent for the treatment or

15 prophylaxis of a disease that includes steps of (a) contacting a sample that contains an altered test protein, and/or portion(s) thereof, and APP, and/or portion(s) thereof, with a test agent, wherein the altered protein is associated with altered A β 42 production, catabolism, processing and/or A β 42 levels; and (b) identifying a candidate agent that restores A β production, catabolism, processing and/or A β levels to substantially that

20 which occurs in the presence of a test protein and/or portion(s) thereof that is not associated with altered A β 42 production, catabolism, processing and/or A β 42 levels without substantially altering the level of one or more other A β peptides. The method can be one wherein the candidate agent restores A β production, catabolism, processing and/or A β levels to substantially that which occurs in the presence of a test protein and/or

25 portion(s) thereof that is not associated with altered A β 42 production, catabolism, processing and/or A β 42 levels without substantially altering the level of A β 40. The method can be one wherein the candidate agent reduces the level of A β 42 and/or increases A β 39 levels. In one embodiment, the step of identifying a candidate agent comprises comparing A β production, catabolism, processing and/or A β levels in a test

30 sample that has been contacted with test agent and a control sample that has not been

- 26 -

contacted with test agent and identifying an agent if A β production, catabolism, processing and/or A β levels in the test sample is such that A β 42 levels differ in the test and control samples and the level of one or more other A β peptides is substantially unchanged in the test and control samples. The level of A β 40 can be substantially
5 unchanged in the test and control samples. The level of A β 42 can be reduced in the test sample relative to the control sample. The level of A β 39 can be increased. In another embodiment, the step of identifying comprises comparing A β production, catabolism, processing and/or A β levels in a test sample that has been contacted with the test agent and a positive control sample and identifying an agent as a candidate agent A β
10 production, catabolism, processing and/or A β levels if A β production, catabolism, processing and/or A β levels in the test and control samples is substantially similar; wherein the positive control sample contains test protein and/or portion(s) thereof that is not associated with altered A β 42 production, catabolism, processing and/or A β 42 levels.

Another method provided herein for identifying a candidate agent for the
15 treatment or prophylaxis of a disease includes steps of contacting a sample that contains an altered test protein, and/or portion(s) thereof, and APP, and/or portion(s) thereof, with a test agent, wherein the altered protein is associated with altered A β production, catabolism, processing and/or A β levels; and identifying a candidate agent that restores A β production, catabolism, processing and/or A β levels to substantially that which
20 occurs in the presence of a test protein and/or portion(s) thereof that is not associated with altered A β production, catabolism, processing and/or A β levels without substantially altering (a) one or more presenilin-dependent activities other than the presenilin-dependent processing of APP, (b) the cleavage and/or processing of a presenilin substrate and/or portion(s) thereof that is other than APP and/or (c) the
25 cleavage and/or processing of LRP and/or portion(s) thereof. For example, the candidate agent can restore A β production, catabolism, processing and/or A β levels to substantially that which occurs in the presence of a test protein and/or portion(s) thereof that is not associated with altered A β production, catabolism, processing and/or A β levels without substantially altering the cleavage and/or processing of Notch, E-cadherin, Erb-B4 and/or
30 portion(s) thereof. The candidate agent can reduce the level of A β 42 and/or increase

- 27 -

A β 39 levels. In one embodiment, the step of identifying a candidate agent comprises comparing A β production, catabolism, processing and/or A β levels in a test sample that has been contacted with test agent and a control sample that has not been contacted with test agent and identifying a candidate agent if A β production, catabolism, processing
5 and/or A β levels in the test sample is such that A β 42 levels differ in the test and control samples and one or more of the following is substantially similar in the test and control samples: (a) one or more presenilin-dependent activities other than the presenilin-dependent processing of APP, (b) the cleavage and/or processing of a presenilin substrate and/or portion(s) thereof that is other than APP and/or (c) the cleavage and/or processing
10 of LRP and/or portion(s) thereof. In a particular embodiment, the step of identifying comprises identifying a candidate agent that restores A β production, catabolism, processing and/or A β levels to substantially that which occurs in the presence of a test protein and/or portion(s) thereof that is not associated with altered A β production, catabolism, processing and/or A β levels without substantially altering the cleavage
15 and/or processing of LRP. The altered protein in these methods can be one that is associated with altered A β 42 production, catabolism, processing and/or A β 42 levels; and the method can include identifying a candidate agent that restores A β production, catabolism, processing and/or A β 42 levels.

In any of the methods provided herein for identifying a candidate agent for the
20 treatment or prophylaxis of a disease, the altered test protein and/or portion(s) thereof can contain a mutation and/or can be altered relative to a wild-type protein, such as a wild-type protein encoded by a predominant allele or that occurs in an organism that exhibits normal A β 42 production, catabolism, processing and/or A β 42 levels. The mutation can be linked to familial Alzheimer's disease. In particular embodiments, the test protein is
25 an APP or a presenilin. If the test protein is an APP, the APP, and/or portion(s) thereof, that is not an altered test protein does not have to be included in the sample. An altered APP or presenilin can be one that is linked to Alzheimer's disease.

The disease can be, for example, an amyloidosis-associated disease, a neurodegenerative disease, and, in particular, Alzheimer's Disease. For any of the
30 methods, the sample can, for example, comprise a cell or organism. The cell can be, for

- 28 -

example, a eukaryotic cell, including a mammalian cell, such as, for example, a rodent or human cell. An organism may be, for example, a non-human transgenic animal.

Also provided are polypeptides comprising a sequence of amino acids that is selectively reactive with A β 42 and preferentially binds to low molecular weight forms of A β 42. The polypeptide can comprise at least one complementarity-determining region (CDR) selected from the group consisting of CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2 or CDR-H3 of antibody A387. In one embodiment, the polypeptide comprises CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2 and CDR-H3 of antibody A387. In another embodiment, the polypeptide comprises at least one CDR selected from the group consisting of amino acids 24-34 of SEQ ID NO:12, amino acids 50-56 of SEQ ID NO:12, amino acids 89-97 of SEQ ID NO:12, amino acids 26-35 of SEQ ID NO:14, amino acids 31-35 of SEQ ID NO:14, amino acids 26-31 of SEQ ID NO:14, amino acids 50-65 of SEQ ID NO:14, amino acids 50-58 of SEQ ID NO:14, and amino acids 98-107 of SEQ ID NO:14. The polypeptide can comprise at least a portion of a variable domain of the light chain or the heavy chain of an A β antibody. In one embodiment, the variable domain is selected from the group consisting of the light chain variable domain of A387, the heavy chain variable domain of A387, a polypeptide with at least 85% identity to the light chain variable domain of A387; a polypeptide with at least 85% identity to the heavy chain variable domain of A387.

The polypeptide can further comprise a scaffold. In one embodiment, the scaffold is a polypeptide scaffold. In one embodiment, the scaffold is a human polypeptide scaffold. In one embodiment, the scaffold is an antibody scaffold. The antibody scaffold can be selected from the group consisting of an Fv fragment scaffold, an Fab fragment scaffold, and a single-chain (scFv) fragment. The polypeptide can further comprise a detectable moiety. The polypeptide can further comprise a clearance domain. The clearance domain can be a ligand for an Fc receptor.

Also provided is a polypeptide, comprising at least one complementarity-determining region (CDR) selected from the group consisting of CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2 or CDR-H3 of antibody A387. In one embodiment, the polypeptide comprises CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2 and CDR-H3 of

- 29 -

antibody A387. In one embodiment, the polypeptide comprises at least one CDR selected from the group consisting of amino acids 24-34 of SEQ ID NO:12, amino acids 50-56 of SEQ ID NO:12, amino acids 89-97 of SEQ ID NO:12, amino acids 26-35 of SEQ ID NO:14, amino acids 31-35 of SEQ ID NO:14, amino acids 26-31 of SEQ ID NO:14, amino acids 50-65 of SEQ ID NO:14, amino acids 50-58 of SEQ ID NO:14, and amino acids 98-107 of SEQ ID NO:14. The polypeptide can also be a chimeric polypeptide. The polypeptide can be an antibody.

The polypeptide can further comprising a clearance domain. The clearance domain can be a ligand for an Fc receptor. The polypeptide can further comprise a detectable moiety. The polypeptide can further comprising a scaffold. In one embodiment, the scaffold comprises a solid support. In another embodiment, the scaffold is a polypeptide scaffold. The scaffold can be a human polypeptide scaffold. The scaffold can be an antibody scaffold. The antibody scaffold can be selected from the group consisting of an Fv fragment scaffold, an Fab fragment scaffold, and a single-chain (scFv) fragment.

The polypeptide can comprise an amino acids 1-95 of SEQ ID NO:12, or a fragment thereof and/or comprises amino acids 1-97 of SEQ ID NO:14, or a fragment thereof. Such polypeptides can further comprise one or more joining regions. In one embodiment, the joining region comprises amino acids 96-107 of SEQ ID NO:12 or amino acids 98-118 of SEQ ID NO:14. In one embodiment, the joining region comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 46, 48, 50, 52, 54, 55, 57, 59, 61, 67, 73, 75, 77, 79, 89 and 91. The polypeptide can further comprising one or more constant regions. The constant region can be a mouse constant region. In one embodiment, the constant region comprises an amino acid sequence selected from the group consisting of SEQ ID NOS:63, 65, 69 and 71. The constant region can also be a human constant region. In one embodiment, the constant region comprises an amino acid sequence selected from the group consisting of SEQ ID NOS:81, 83, 85 and 87. The polypeptide can comprise the amino acid sequence of SEQ ID NO:97 and/or SEQ ID NO:98.

The polypeptide can be specifically reactive with at least one A β . In one

- 30 -

embodiment, A β is A β 42. In one embodiment, the polypeptide binds A β 42 without substantially binding other A β peptides.

Also provided is a polypeptide comprising at least one complementarity-determining region (CDR) selected from the group consisting of CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2 or CDR-H3 of antibody B436. In one embodiment, the polypeptide comprises CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2 and CDR-H3 of antibody B436. In one embodiment, the polypeptide comprises at least one CDR selected from the group consisting of amino acids 24-39 of SEQ ID NO:16, amino acids 55-61 of SEQ ID NO:16, amino acids 94-102 of SEQ ID NO:16, amino acids 26-35 of SEQ ID NO:18, amino acids 31-35 of SEQ ID NO:18, amino acids 26-31 of SEQ ID NO:18, amino acids 50-66 of SEQ ID NO:18, amino acids 50-59 of SEQ ID NO:18, and amino acids 99-103 of SEQ ID NO:18. The polypeptide can be a chimeric polypeptide. The polypeptide can be an antibody.

The polypeptide can further comprise a scaffold. In one embodiment, the scaffold comprises a solid support. In one embodiment, the scaffold is a polypeptide scaffold. The scaffold can be a human polypeptide scaffold. The scaffold can be an antibody scaffold. The antibody scaffold can be selected from the group consisting of an Fv fragment scaffold, an Fab fragment scaffold, and a single-chain (scFv) fragment. The polypeptide can be specifically reactive with at least one A β peptide.

The polypeptide can further comprise a clearance domain. The clearance domain can be a ligand for an Fc receptor. The polypeptide can further comprise a detectable moiety.

The polypeptide can comprise amino acids 1-100 of SEQ ID NO:16, or a fragment thereof and/or comprises amino acids 1-98 of SEQ ID NO:18, or a fragment thereof. Such polypeptides can further comprise one or more joining regions. In one embodiment, the joining region can comprise amino acids 101-112 of SEQ ID NO:16 or amino acids 99-114 of SEQ ID NO:18. In one embodiment, the joining region comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 46, 48, 50, 52, 54, 55, 57, 59, 61, 67, 73, 75, 77, 79, 89 and 91. The polypeptide can further comprising one or more constant regions. In one embodiment, the constant region is a

- 31 -

mouse constant region. The mouse constant region can comprise an amino acid sequence selected from the group consisting of SEQ ID NOS:63, 65, 69 and 71. In one embodiment, the constant region is a human constant region. The human constant region can comprise an amino acid sequence selected from the group consisting of SEQ ID NOS:81, 83, 85 and 87. The polypeptide can comprise the amino acid sequence of SEQ ID NO:99 and/or SEQ ID NO:100.

Also provided are nucleic acid molecules encoding polypeptides provided herein. In one embodiment, the nucleic acid molecule encodes a polypeptide comprising a sequence of amino acids that is selectively reactive with A β 42 and preferentially binds to low molecular weight forms of A β 42. In one embodiment, the nucleic acid molecule encodes a polypeptide comprising at least one complementarity-determining region (CDR) selected from the group consisting of CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2 or CDR-H3 of antibody A387. Also provided are nucleic acid molecules encoding a polypeptide comprising at least one complementarity-determining region (CDR) selected from the group consisting of CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2 or CDR-H3 of antibody B436. Also provided are kits comprising the polypeptides described herein.

Further provided are methods for assessing the presence or amount of A β in a sample, comprising contacting a polypeptide provided herein with the sample under conditions whereby a complex is formed between the polypeptide and A β , and assessing the presence or amount of the complex in the sample, and thereby determining the presence or amount of A β in the sample. The sample can be selected from the group consisting of a cell extract, extracellular medium, plasma, cerebrospinal fluid and brain. The presence or amount of the complex can be assessed using an enzyme-linked immunosorbent assay (ELISA).

Also provided are methods comprising administering to a subject a polypeptide provided herein. In one embodiment, the polypeptide comprises at least one complementarity-determining region (CDR) selected from the group consisting of CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2 or CDR-H3 of antibody A387. In one embodiment, the polypeptide is selectively reactive with A β 42 and preferentially binds to

- 32 -

low molecular weight forms of A β 42. In one embodiment, the polypeptide comprises at least one complementarity-determining region (CDR) selected from the group consisting of CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2 or CDR-H3 of antibody B436. In one embodiment, the subject has, or is at risk of developing, a disease associated with accumulation of A β . The disease can be Alzheimer's disease.

Also provided are methods of binding A β comprising administering to a subject a polypeptide provided herein to bind A β . In one embodiment, the polypeptide comprises at least one complementarity-determining region (CDR) selected from the group consisting of CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2 or CDR-H3 of antibody A387 wherein the polypeptide is specifically reactive with at least one A β peptide. In one embodiment, the polypeptide is selectively reactive with A β 42 and preferentially binds to low molecular weight forms of A β 42. In one embodiment, the polypeptide comprises at least one complementarity-determining region (CDR) selected from the group consisting of CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2 or CDR-H3 of antibody B436, wherein the polypeptide is specifically reactive with at least one A β peptide. In one embodiment, the subject has, or is at risk of developing, a disease associated with accumulation of A β . The disease can be Alzheimer's disease.

Also provided are methods of reducing A β level in an subject, comprising administering to the subject an effective amount of a polypeptide provided herein to reduce the level of at least one A β peptide. In one embodiment, the polypeptide comprises at least one complementarity-determining region (CDR) selected from the group consisting of CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2 or CDR-H3 of antibody A387 wherein the polypeptide is specifically reactive with at least one A β peptide. In one embodiment, the polypeptide is selectively reactive with A β 42 and preferentially binds to low molecular weight forms of A β 42. In one embodiment, the polypeptide comprises at least one complementarity-determining region (CDR) selected from the group consisting of CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2 or CDR-H3 of antibody B436, wherein the polypeptide is specifically reactive with at least one A β peptide. In one embodiment, the subject has, or is at risk of developing, a disease associated with accumulation of A β . The disease can be Alzheimer's disease. In one

- 33 -

embodiment, the level of at least one A β peptide in blood or plasma is reduced. In one embodiment, the level at least one A β peptide in brain is reduced.

Also provided are methods for identifying an agent that modulates A β levels, comprising comparing the levels of bound A β binding protein in a test sample contacted
5 with the test agent and a control sample not contacted with the test agent; and identifying an agent as an agent that modulates A β levels if the levels of bound A β binding protein differ in the test and control samples; wherein the sample comprises APP or portion(s) thereof. The A β binding protein comprises a polypeptide provided herein. In one
10 embodiment, the polypeptide comprises at least one complementarity-determining region (CDR) selected from the group consisting of CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2 or CDR-H3 of antibody A387 wherein the polypeptide is specifically reactive with at least one A β peptide. In one embodiment, the polypeptide is selectively reactive with A β 42 and preferentially binds to low molecular weight forms of A β 42. In one embodiment, the polypeptide comprises at least one complementarity-determining
15 region (CDR) selected from the group consisting of CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2 or CDR-H3 of antibody B436, wherein the polypeptide is specifically reactive with at least one A β peptide.

Also provided are methods for identifying an agent that modulates A β 42 levels, comprising, comparing the levels of bound A β binding protein in a test sample contacted
20 with the test agent and a control sample not contacted with the test agent; and identifying an agent as an agent that modulates A β 42 levels if the levels of bound A β binding protein differ in the test and control samples; wherein the sample comprises APP or portion(s) thereof; and the A β binding protein comprises a sequence of amino acids selected from the group consisting of amino acids 1-50, 1-60, 1-70, 1-80, 1-90, 1-91, 1-92, 1-93, 1-94,
25 1-95, 1-96 and 1-97 of SEQ ID NO: 12 and 1-50, 1-60, 1-70, 1-80, 1-90, 1-91, 1-92, 1-93, 1-94, 1-95, 1-96 and 1-97 of SEQ ID NO: 14 and any amino acid sequences containing modifications of these amino acid sequences that retain the A β binding properties of an antibody comprising one or both of the amino acid sequences set forth as amino acids 1-95 of SEQ ID NO: 12 and amino acids 1-97 of SEQ ID NO: 14.

30 Further provided herein are methods in the treatment or prophylaxis of disease

- 34 -

involving or characterized by A β and/or specific A β forms. In one embodiment, the method includes a step of administering a polypeptide provided herein to a subject having such a disease or disorder or predisposed to such a disease or disorder. In one embodiment, the disease is Alzheimer's disease. In one embodiment, A β 42 levels are
5 modulated. In one embodiment, the polypeptide is an A β binding protein or A β antibody. In one embodiment, the polypeptide comprising a sequence of amino acids that is selectively reactive with A β 42 and preferentially binds to low molecular weight forms of A β 42.

DETAILED DESCRIPTION

10 A. DEFINITIONS

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of ordinary skill in the art to which this invention belongs. All patents, applications, published applications and other
15 publications and sequences from GenBank and other data bases referred to herein are incorporated by reference in their entirety. Where reference is made to a URL or other such identifier or address, it is understood that such identifiers can change and particular information on the internet can come and go, but equivalent information can be found by searching the internet. Reference thereto evidences the availability and public dissemination of such information.

20 As used herein, "Alzheimer's disease" or "AD" refers to a group of visible, detectable or otherwise measurable properties characteristic of AD. Exemplary properties include, but are not limited to, dementia, aphasia (language problems), apraxia (complex movement problems), agnosia (problems in identifying objects), progressive memory impairment, disordered cognitive function, altered behavior, including paranoia,
25 delusions and loss of social appropriateness, progressive decline in language function, slowing of motor functions such as gait and coordination in later stages of AD, amyloid-containing plaques which are foci of extracellular amyloid- β (A β) protein deposition with dystrophic neurites and associated axonal and dendritic injury and microglia expressing surface antigens associated with activation (e.g., CD45 and HLA-DR), diffuse
30 ("preamyloid") plaques and neuronal cytoplasmic inclusions such as neurofibrillary

- 35 -

tangles containing hyperphosphorylated tau protein or Lewy bodies (containing α -synuclein). Standardized clinical criteria for the diagnosis of AD have been established by NINCDS/ADRDA (National Institute of Neurological and Communicative Disorders and Stroke/Alzheimer's Disease and Related Disorders Association) (McKhann *et al.* (1984) *Neurology* 34:939-944). The clinical manifestations of AD as set forth in these criteria are included within the definition of AD. For example, dementia may be established by clinical exam and documented by any of several neuropsychological tests, including the Mini Mental State Exam (MMSE) (Folstein and McHugh (1975) *J. Psychiatr. Res.* 12:196-198; Cockrell and Folstein (1988) *Psychopharm. Bull.* 24:689-692), the Blessed Test (Blessed *et al.* (1968) *Br. J. Psychiatry* 114:797-811), and the Alzheimer's Disease Assessment Scale-Cognitive (ADAS-COG) Test (Rosen *et al.* (1984) *Am. J. Psychiatry* 141:1356-1364; Weyer *et al.* (1997) *Int. Psychogeriatr.* 9:123-138; and Ihl *et al.* (2000) *Neuropsychobiol.* 4:102-107).

As used herein, "amyloidosis" refers to a condition characterized by the presence of amyloid. Amyloid refers to a group of diverse but specific protein deposits observed in a number of different diseases. An example of an amyloid deposit is the β -amyloid plaque that is a defining pathological hallmark of Alzheimer's disease. The major protein component of the β -amyloid plaque is the A β peptide which is derived from processing of amyloid precursor protein (APP). Amyloid deposits, though diverse in their occurrence, can share some common morphologic properties. Many stain with specific dyes (*e.g.*, Congo red), and have a characteristic red-green birefringent appearance in polarized light after staining. Some share ultrastructural features and common x-ray diffraction and infrared spectra. Amyloidosis can be classified clinically as primary, secondary, familial and/or isolated. Primary amyloid appears *de novo* without any preceding disorder. Secondary amyloid is that form which appears as a complication of a previously existing disorder. Familial amyloid is a genetically inherited form found in particular geographic populations. Isolated forms of amyloid are those that tend to involve a single organ system.

An amyloidosis-associated disease is a disease involving accumulation of amyloid. Such diseases include, but are not limited to, AD, Down's syndrome, familial

- 36 -

5 amyloid polyneuropathy, familial amyloid cardiomyopathy (Danish type), isolated cardiac amyloid, amyloid angiopathy, systemic senile amyloidosis, idiopathic (primary) amyloidosis, reactive (secondary) amyloidosis, familial amyloidosis of Finnish type, and hereditary cerebral hemorrhage with amyloidosis-Dutch type (HCHWA-D) and Icelandic type.

As used herein, "amyloid precursor protein" or "APP" refers to a protein containing several characteristic domains, including a heparin-binding site, zinc- and copper-binding domains, a trophic domain containing an amino acid sequence (RERMS) that promotes fibroblast growth and a protease inhibitor domain for the matrix metalloprotease gelatinase A. Multiple isoforms of APP exist, typically distinguished by the number of amino acids in the particular isoform. Generally, most isoforms of APP are approximately 100 kD in molecular weight. Isoforms of APP include, for example, APP770 (which also contains a sequence homologous to the Kunitz family of serine protease inhibitors and a sequence homologous to the MRC OX-2 antigen), APP751 (the most abundant APP isoform in non-neuronal tissues), APP714, APP695 (the most abundant form in the brain), L-APP752, L-APP733, L-APP696, L-APP677, APP563 and APP365. All of the above-mentioned isoforms of APP, with the exception of APP563 and APP365, are transmembrane proteins that contain a single membrane-spanning domain and a long N-terminal extracellular (about two-thirds of the protein) and C-terminal cytoplasmic regions. APP563 and APP365 lack a transmembrane domain and are secreted. Examples of amino acid sequences for some of the APP isoforms are provided in SEQ ID NOs: 2 (APP770), 28 (APP751) and 30 (APP695). In addition, several mutations of the APP gene demonstrated in families with AD and other amyloidosis-associated diseases yield APP forms with varying amino acid sequences. Mutations of APP include those that result in a Val to Gly substitution at position 717 (V717G) of APP770 (the "London variant"), the "Swedish variant" double mutation at amino acid positions 670 and 671, with reference to the APP770 isoform, or positions 595 and 596, with reference to the APP695 isoform, in which a lysine is substituted with an asparagine and a methionine is substituted with a leucine, respectively, and a mutation at position 693 of the APP770 isoform that is associated with hereditary cerebral

- 37 -

hemorrhage with amyloidosis-Dutch type (HCHWA-D). Unless a specific isoform is specified, APP when used herein generally refers to any and all isoforms of APP.

As used herein, "cleavage" when used with reference to a substrate, *e.g.*, a protein, polypeptide, peptide, or fragment(s) thereof, refers to an alteration in the substrate structure. The alteration can be one resulting from, for example, an alteration, elimination or reduction of one or more interactions between elements within the substrate. In one example, if the substrate is a protein (polypeptide or peptide), cleavage of the substrate can be the degradation of the protein by a loss of one or more amino acids from the protein. The protein substrate, may, for example, be degraded into two or more fragments, each of which contains less than all the amino acids that the substrate contained. For instance, the processing of a larger precursor protein to yield a smaller mature protein can involve protein cleavage. Such cleavage can be, for example, the result of the hydrolysis of one or more peptide bonds in the protein. Thus, cleavage includes proteolytic cleavage of protein substrates. An alteration of a substrate structure due to cleavage (*e.g.*, the particular one or fragments generated upon cleavage of a protein substrate) can provide information relating to the types of compositions (*e.g.*, protease or proteolytic enzymes) and/or conditions or activities to which the substrate has been exposed.

As used herein, "processing" with reference to a protein, polypeptide or peptide refers to any post-translational modifications or alterations of the protein, polypeptide or peptide, such as may occur in maturation, degradation and/or clearance of such a molecule in a cell, and/or any post-translational packaging or transport of such a molecule through a pathway or process, such as a secretory pathway, uptake/internalization process, exo- or endocytosis, sequestration (*e.g.*, into a vesicle or endosome or lysosome) and clearance. In one example of processing, a protein, polypeptide or peptide can undergo cleavage, for example, to yield an active peptide from a larger inactive precursor protein, to liberate a functional fragment or peptide, such as a signaling peptide, or to degrade/digest a protein, polypeptide or peptide.

As used herein, "portion" and "fragment" are used interchangeably with reference to a protein, polypeptide or peptide and refer to a protein, polypeptide or peptide with a

- 38 -

primary structure that is less than or smaller than that of the protein, polypeptide or peptide of which it is a portion or fragment. For example, a fragment or portion of a protein can be a peptide generated upon cleavage of a larger precursor protein.

As used herein, "amyloid- β peptide" or " $A\beta$ " refers to a peptide such as (a) a
5 peptide that results from processing or cleavage of an APP and that is amyloidogenic, (b) one of the peptide constituents of β -amyloid plaques, (c) the 43-amino acid sequence set forth in SEQ ID NO: 4 or a fragment or portion thereof, and including substantially homologous sequences and/or (d) a fragment or portion of a peptide as set forth in (a) or (b). $A\beta$ can also be referred to as β AP, $A\beta$ P or β A4. $A\beta$ peptides derived from
10 proteolysis of APP generally are ~4.2 kD proteins and are typically 39 to 43 amino acids in length (see, *e.g.*, SEQ ID NO: 4 showing the 43-amino acid sequence of an $A\beta$ peptide), depending on the carboxy-terminal end-point, which exhibits heterogeneity. However, $A\beta$ peptides containing less than 39 amino acids, *e.g.*, $A\beta$ 39, $A\beta$ 38, $A\beta$ 37 and $A\beta$ 34, also can occur. $A\beta$ peptides can be produced in an amyloidogenic APP processing
15 pathway in which APP is cleaved by β -secretase (BACE) and one or more γ -secretase activities. $A\beta$ peptides include those that begin at position 672 of APP770 (see SEQ ID NO: 2). Generally, as used herein, " $A\beta$ peptide" includes any and all $A\beta$ peptides, unless the amino acid residues are specified, such as, for example, 1-42 ($A\beta$ 42), 1-40 ($A\beta$ 40), 1-39 ($A\beta$ 39), 1-38 ($A\beta$ 38), 1-37 ($A\beta$ 37), 1-34 ($A\beta$ 34) and others.

20 As used herein "at least one $A\beta$ peptide" refers to one or more species or sequence of amino acids of $A\beta$. For example, at least one $A\beta$ peptide can be $A\beta$ 42, $A\beta$ 40, $A\beta$ 39, $A\beta$ 38, $A\beta$ 34, and combinations thereof.

As used herein, "form of $A\beta$ " or " $A\beta$ form" refers to the conformational state of $A\beta$, for example monomers, oligomers such as dimers, trimers, pentamers, low molecular
25 weight and high molecular weight oligomers of $A\beta$. Forms of $A\beta$ also include aggregates, fibrils, tangles, and soluble $A\beta$. As used herein, "low molecular weight forms of $A\beta$ " refers to monomers and low molecular weight oligomers of $A\beta$, including oligomers containing from about two to about 10 molecules of $A\beta$. As used herein, "high molecular weight forms of $A\beta$ " refers to high molecular weight forms of $A\beta$ such
30 as aggregates of 50 or more $A\beta$ molecules.

- 39 -

As used herein, "A β misregulation" refers to altered, abnormal or impaired A β regulation. For example, A β misregulation can be imbalances or disturbances in intracellular and/or secreted levels such as may result from altered A β production, clearance or degradation in a cell.

5 As used herein, "cellular" or "cell-associated" with reference to a molecule, such as, for example, a protein or peptide, refers to a molecule that is located within a cell (*e.g.*, in the cytoplasm or an intracellular organelle or vesicle) and/or at least partially associated with or in a cell membrane (*e.g.*, the plasma membrane or an intracellular membrane).

10 As used herein, "low-density lipoprotein receptor-related protein (LRP)" refers to a protein homologous to LRP_s, which have been identified and described for a number of species, including several mammalian species. An example of an amino acid sequence of an LRP is provided in SEQ ID NO: 10. LRP proteins, which are discussed below in more detail, generally are cell surface receptors that bind and internalize a number of
15 diverse extracellular ligands, including apolipoprotein E (apoE), α 2-macroglobulin (α 2M), APP, tissue-type plasminogen activator (tPA) and lactoferrin, for degradation by lysosomes. LRP expression is widespread; however, it is most highly expressed in the liver, brain and placenta. LRP is a member of the low-density lipoprotein receptor (LDLR) family. The extracellular region of receptors in this family contains several
20 structural modules which include ligand-binding repeats of ~40 amino acids (including six cysteine residues forming three disulfide bonds), epidermal growth factor (EGF) precursor repeats (each also containing six cysteine residues), and modules with a consensus tetrapeptide (YWTD). In addition to these modules, these receptors contain a single transmembrane domain and a relatively short cytoplasmic tail with endocytosis
25 signals and elements for interaction with cytoplasmic adaptor and scaffold proteins (*e.g.*, Dab, FE65, c-jun N-terminal kinase interacting proteins (JIPs) and postsynaptic density protein PSD-95) for mediating signal transduction.

As used herein, a "composition of low-density lipoprotein receptor-related protein (LRP)" refers to the make-up of LRP. The LRP may be LRP that is present anywhere,
30 for example, in an analysis mixture, including an assay medium in which an analysis is

- 40 -

performed, an extracellular medium, or a cell membrane, lysate or extract. For example, a composition of LRP refers to the overall combination of any intact LRP protein(s), fragments thereof, sizes thereof, ratios and amounts thereof.

As used herein, "presenilin" refers to a protein homologous to the presenilin 1 (PS1) or presenilin 2 (PS2) proteins, and/or fragment(s) thereof, that have been identified and described for a number of species, including several mammalian species. Presenilins show a high degree of conservation between species, particularly of the hydrophobic structure. Examples of amino acid sequences of PS1 and PS2 proteins are provided in SEQ ID NOs: 6 and 8, respectively and in PCT Application Publication No. WO96/34099. Presenilin proteins generally are polytopic membrane proteins that can possess two or more aspartic acid residues within adjacent predicted transmembrane segments. Many presenilins possess protease-associated domains and are involved in a catalytic complex having catalytic activity. Presenilins can undergo proteolytic processing which can generate fragments, such as, for example, an ~35-kD N-terminal fragment and an ~20-25 kD C-terminal fragment. In vivo, the majority of detectable presenilin appears in the form of N- and C-terminal fragments that are tightly regulated and form a stable complex after processing. Thus, as used herein, "presenilin" refers to any full-length presenilin protein, presenilin proteins encoded by allelic and splice variants, and any fragments thereof, including biologically active fragments and functional units.

As used herein, "presenilin activity" or "presenilin-dependent activity" refers to an activity, such as a biological event or process, that is directly or indirectly influenced by a presenilin protein. An activity can be, for example, any biological, chemical, biochemical or molecular activity, including, but not limited to, interaction between molecules, such as binding between a protein or peptide and another molecule, a chemical reaction, *e.g.*, hydrolysis, and a cellular event, *e.g.*, secretion, endocytosis, signaling, molecular trafficking. A presenilin-dependent activity is influenced by a presenilin in such a way that the activity differs in the presence and absence of a presenilin. The difference can be, for example, a modification or alteration in the activity or a complete or near complete elimination of the activity. In a particular example, a

- 41 -

presenilin-dependent activity is an enzymatic activity. One such presenilin-dependent enzymatic activity is a presenilin-dependent proteolytic processing of APP, *e.g.*, γ -secretase cleavage of APP. Other presenilin-dependent enzymatic activities include, but are not limited to, cleavage of LRP, Notch, E-cadherin and Erb-B4.

5 As used herein, "presenilin substrate," "substrate for presenilin activity" and/or "substrate for presenilin-dependent enzyme activity" refers to a peptide, polypeptide, protein or fragment(s) thereof that is altered (*e.g.*, proteolytically processed, at least in part) in a presenilin-dependent manner. Thus, for example, in the case of a presenilin substrate that is altered by proteolytic processing of the substrate, if presenilin is absent,
10 or presenilin activity is inhibited or reduced, the proteolytic processing of the presenilin substrate is altered, for example by an alteration in the levels and/or composition of fragments generated from the substrate, relative to the proteolytic processing of the substrate that occurs in the presence of normal (*e.g.*, wild-type) presenilin activity. Generally, a presenilin substrate can contain about one transmembrane domain, an
15 ectodomain that is released or shed into the extracellular medium, and/or an intracellular domain. Exemplary presenilin substrates include, but are not limited to APP, LRP, Notch, TrkB, APLP2, hIre1 α , E-cadherin and Erb-B4.

 As used herein, "C-terminal fragment (CTF)" refers to a fragment of a protein that results from cleavage of the protein by a presenilin-dependent activity. For example,
20 an LRP-CTF refers to a C-terminal fragment of LRP. When an LRP composition is assessed, for example, it can be evaluated to determine if any fragment(s) indicative of presenilin-dependent cleavage (or altered presenilin-dependent cleavage) of LRP is/are present and/or the level of any such fragment(s) produced. A presenilin-dependent cleavage described herein occurs within the C-terminal portion of LRP and within the β
25 chain. Thus, a presenilin-dependent cleavage of LRP can be one, for example, that occurs in the C-terminal portion of LRP at a position C-terminal to amino acid position 3925 of SEQ ID NO: 10 (or of the amino acid sequence provided as GenBank Accession No. Q07954). The presenilin-dependent cleavage of LRP can be one that occurs within the sequence of the last approximately 580, 500, 450, 400, 350, 300, 250, 200, 150, 100,
30 50, 25, or less amino acids of LRP. The presenilin-dependent cleavage can be one that

- 42 -

occurs C-terminal to the extracellular portion of the β chain (i.e., approximately amino acids 3944-4420 of SEQ ID NO: 10 or of the amino acid sequence provided as GenBank Accession No. Q07954); thus, C-terminal to amino acid 4420 of SEQ ID NO:10. The presenilin-dependent cleavage of LRP can be one that occurs near or within the region of the LRP protein that extends from a point located intracellularly and adjacent to the cytoplasmic face of the cell membrane to a point located within the cell membrane. Such a presenilin-dependent cleavage of LRP can be one that generates a soluble intracellular peptide containing the extreme C-terminus of LRP and a membrane-associated peptide containing amino acid sequence of the transmembrane region of LRP, particularly the more C-terminal region of the transmembrane segment of LRP. Such fragments of LRP can be referred to as LRP-CTFs. Any LRP fragments generated by such presenilin-dependent activities have a molecular weight that is less than that of the β chain of LRP (β chain molecular weight is approximately 85-90 kD, or approximately 67 kD after deglycosylation with *N*-glycosidase F) and are encompassed by the term LRP-CTFs. Similarly, characteristic C-terminal fragments of APP are produced upon exposure to an a presenilin-dependent activity.

As used herein, "normal" with reference to a protein refers to a protein which performs its usual or normal physiological role and which is not causative of a disease or pathogenic condition. A normal gene or coding sequence is also one that is not causative of a disease or pathogenic condition and may encode a normal protein. The term normal is generally synonymous with wild-type. For any given gene, or corresponding protein, a number of normal allelic variants may exist, none of which is associated with the development of a pathogenic condition or disease.

As used herein, "mutant" with reference to a protein refers to a protein which does not perform its usual or normal physiological role, *e.g.*, it may be dysfunctional, and which can be associated with a disease or pathogenic state. A mutant gene generally is one that contains an alteration relative to a normal or wild-type gene such that it has altered function (*e.g.*, regulation or encoding of a mutant protein).

As used herein, "assess" and variations thereof refer to any type of evaluation, determination, observation, identification, detection, characterization and measurement,

- 43 -

whether quantitative, qualitative, comparative or relative.

As used herein, "determining the level of", "assessing the level of" and variations thereof with reference to a substance, such as, for example a peptide, protein or fragment thereof, can be determining the presence or absence of the substance and/or making a
5 more quantitative assessment of level or amount of the substance.

As used herein, the term "polypeptide" is used interchangeably with the term "protein" and includes peptides of 2 or more amino acids. A polypeptide can be a single polypeptide chain, or to two or more polypeptide chains that are held together by non-covalent forces, by disulfide cross-links, or by other linkers (e.g. peptide linkers). Thus,
10 a single heavy or light chain of an antibody, or an antibody fragment containing all or part of both heavy and light chains of an antibody, no matter how the chains are associated or joined, are exemplary molecules that are included within the term "a polypeptide." A polypeptide can contain non-proteinaceous components, such as sugars, lipids, detectable labels or therapeutic moieties. A polypeptide can be derivatized by
15 chemical or enzymatic modifications (e.g. by replacement of hydrogen by an alkyl, acyl, or amino group; esterification of a carboxyl group with a suitable alkyl or aryl moiety; alkylation of a hydroxyl group to form an ether derivative; phosphorylation or dephosphorylation of a serine, threonine or tyrosine residue; or N- or O-linked glycosylation) or can contain substitutions of an L-configuration amino acid with a D-
20 configuration counterpart.

As used herein, the term "chimeric polypeptide" refers to a polypeptide that contains amino acid residues from derived from two or more polypeptides or from one polypeptide but joined in different order from the original polypeptide. For example, a chimeric polypeptide can contain residues from related polypeptides from two or more
25 species (e.g. CDR sequences from a mouse immunoglobulin (Ig), and a scaffold portion from a human Ig; or variable region residues from a mouse Ig, and constant region residues from a human Ig). A chimeric polypeptide also can contain residues from two or more unrelated polypeptides from the same or different species (e.g. CDR sequences from an Ig, and scaffold sequences from a lipocalin or Fn3 polypeptide).

30 As used herein, "antibody" refers to an immunoglobulin, whether natural or

- 44 -

partially or wholly synthetically produced, including any derivative thereof that retains the specific binding ability of the antibody. Hence antibody includes any protein having a binding domain that is homologous or substantially homologous to an immunoglobulin binding domain. Antibodies include members of any immunoglobulin chains, including IgG, IgM, IgA, IgD and IgE. As used herein, the term "antibody" includes, but is not limited to, polyclonal antibodies, affinity-purified polyclonal antibodies, monoclonal antibodies, antibody fragments and antigen-binding fragments. Genetically engineered intact antibodies or fragments, such as chimeric antibodies, Fv fragments, single chain antibodies and the like, as well as synthetic antigen-binding peptides and polypeptides, are also included.

As used herein, "antibody fragment" refers to any derivative of an antibody that is less than full-length, retaining at least a portion of the full-length antibody's specific binding ability. Examples of antibody fragments include, but are not limited to, Fab, Fab', F(ab)₂, single-chain Fvs (scFv), FV, dsFv diabody and Fd fragments. The fragment can include multiple chains linked together, such as by disulfide bridges. An antibody fragment generally contains at least about 50 amino acids and typically at least 200 amino acids.

As used herein, an "Fv antibody fragment" is composed of one variable heavy domain (V_H) and one variable light domain linked by noncovalent interactions.

As used herein, a "dsFV" refers to an Fv with an engineered intermolecular disulfide bond, which stabilizes the V_H-V_L pair.

As used herein, an "F(ab)₂ fragment" is an antibody fragment that results from digestion of an immunoglobulin with pepsin at pH 4.0-4.5; it can be recombinantly expressed to produce the equivalent fragment.

As used herein, "Fab fragments" are antibody fragments that result from digestion of an immunoglobulin with papain; they can be recombinantly expressed to produce the equivalent fragment.

As used herein, "scFvs" refer to antibody fragments that contain a variable light chain (V_L) and variable heavy chain (V_H) covalently connected by a polypeptide linker in any order. The linker is of a length such that the two variable domains are bridged

- 45 -

without substantial interference. Included linkers are (Gly-Ser)_n residues with some Glu or Lys residues dispersed throughout to increase solubility.

As used herein, "diabodies" are dimeric scFv; diabodies typically have shorter peptide linkers than scFvs, and they generally dimerize.

5 As used herein, the term "complementarity determining region" or "CDR" (also known as a "hypervariable region") refers to a region of an Ig molecule that varies greatly in amino acid sequence relative to flanking Ig sequences. The length and conformation of CDRs vary among Igs, but generally CDRs form short loops supported by a sandwich of two antiparallel beta-sheets. Three CDRs, designated CDR-L1, CDR-L2 and CDR-
10 L3, are present in the variable region of an immunoglobulin light chain, and three CDRs, designated CDR-H1, CDR-H2 and CDR-H3, are present in the variable region of an immunoglobulin heavy chain. Each CDR generally contains at least one, and often several, amino acids residues that make contact with antigen, but all six CDRs are not necessarily required to maintain the binding specificity of an antibody.

15 As used herein, a "scaffold" refers to any structure that forms a conformationally stable structural support, or framework, which is able to display one or more sequences of amino acids (e.g. CDRs, a variable region, a binding domain) in a localized surface region. A scaffold can be a naturally occurring polypeptide or polypeptide "fold" (a structural motif), or can have one or more modifications, such as additions, deletions or
20 substitutions of amino acids, relative to a naturally occurring polypeptide or fold. Exemplary modifications to a polypeptide that render it suitable for use as a scaffold include but are not limited to, deletions of those regions that form binding loops in the naturally-occurring molecule (e.g. deletions of the naturally-occurring CDRs); deletions of those regions that are unnecessary for structural integrity of the fold; substitutions of
25 amino acids that flank the loop regions with residues that improve the properties of the polypeptide (such as improved affinity, specificity, or solubility; reduced immunogenicity, etc.); addition of detectable sequences, such as epitope tags. A scaffold can be derived from a polypeptide of any species (or of more than one species), such as a human, other mammal, other vertebrate, invertebrate, plant, bacteria or virus. A scaffold
30 can also be a solid support, such as a membranes, filters, chips, slides, wafers, fibers,

- 46 -

magnetic or nonmagnetic beads, gels, tubing, plates, polymers, microparticles and capillaries, which is able to display one or more amino acid sequences (e.g. CDRs) in a localized surface region.

As used herein, the term "human polypeptide scaffold" refers to a polypeptide scaffold that is derived from a human polypeptide or has been engineered to resemble a human polypeptide. An example of a human polypeptide scaffold is a human antibody scaffold, which is used in a humanized antibody.

As used herein, the term "antibody scaffold" refers to a scaffold of an antibody that contains all or part of an immunoglobulin. Exemplary antibody scaffolds include whole antibodies, and fragments thereof, such as Fv fragments (which can or can not contain an introduced disulfide bond), Fab fragments, Fab' fragments, F(ab')₂ fragments, and single-chain scFv fragments. Antibody scaffolds also include all or part of an Ig heavy chain variable region, and all or part of an Ig light chain variable region.

As used herein, the term "clearance domain" refers to a domain that directly or indirectly mediates enhanced clearance of a polypeptide from the circulation. Thus, a polypeptide described herein as containing a "clearance domain" will have a shorter half-life in the circulation, alone and/or when bound to A β , than a polypeptide without such a domain.

As used herein, an "A β antibody" refers to an immunoglobulin, whether natural or partially or wholly synthetically produced, including any derivative thereof that is specifically reactive with at least one A β .

As used herein, an "A β binding protein" refers to a polypeptide, peptide or protein that is specifically reactive with at least one A β peptide. An A β binding protein can be an A β antibody or fragment(s) thereof. A β proteins also include chimeric polypeptides. For example, an A β binding protein can be a chimeric polypeptide that has the ability to bind A β displayed in a scaffold. An A β binding protein can also be derived de novo by screening for peptides, polypeptides and proteins that have the ability to bind at least one A β .

As used herein, "grafting" with respect to polypeptides refers to the construction of a chimeric polypeptide by covalently joining a peptide, protein or domain of a protein

- 47 -

to a scaffold.

As used herein, “operatively linked” (or, sequences that are in “operative association”) indicates that the recited nucleotide sequences are positioned such that there is a functional relationship between the sequences in the context of transcription.

5 For example, an A β binding protein nucleotide sequence, a promoter sequence and a reporter sequence can be in operative association if transcription of the reporter nucleic acid sequence can occur under control of the promoter sequence as modulated by the effect of the A β binding protein nucleotide sequence. When the A β binding protein nucleotide sequence comprises the promoter, the sequences can be in operative
10 association if transcription of the reporter nucleic acid sequence can occur under control of the A β binding protein nucleotide sequence. Two sequences that are “operatively linked” are not necessarily contiguous.

As used herein, an “expression construct” refers to a nucleotide sequence with the capacity to express an mRNA or protein. Generally, expression constructs have a
15 sequence of nucleotides encoding the mRNA and/or protein to be expressed, operatively linked to a promoter sequence.

As used herein a “detectable moiety” refers to a molecule that can be detected by visible, enzymatic, physical or chemical means. Detectable moieties include, but are not limited to, reporter genes or fragments thereof, enzymes or portions thereof and
20 radiolabels. Exemplary detectable moieties include fluorescent proteins such as green, red and blue fluorescent proteins, β -galactosidase, alkaline phosphatase and radiolabels such as ^{125}I , ^{131}I , ^{213}Bi , ^{99}Tc , ^{111}In , ^{90}Y , and ^{32}P . Detectable moieties also include moieties that can be detected physical means such as detection of molecular weight by mass spectrometry and tags that can be detected such as a His₆ tag for metal binding or
25 an epitope tag for antibody recognition.

As used herein, “humanized antibodies” refer to antibodies that are modified to include human sequences of amino acids so that administration to a human does not provoke an immune response, or provokes a milder immune response than a non-humanized antibody. Methods for preparation of such antibodies are known. For
30 example, to produce such antibodies, the encoding nucleic acid in the hybridoma or other

- 48 -

prokaryotic or eukaryotic cell, such as an *E. coli* or a CHO cell, that expresses the monoclonal antibody is altered by recombinant nucleic acid techniques to express an antibody in which the amino acid composition is based on human antibodies.

As used herein, "specifically reactive," "specificity," "selectivity," "selective" and variations thereof in the context of an antibody binding an antigen refers to the degree of affinity an antibody has for a target antigen and the degree of discrimination between the target antigen and other, chemically similar structures. Antibodies and proteins, such as A β binding proteins, are determined to be specifically reactive if: 1) they exhibit a threshold level of binding affinity, and/or 2) they do not significantly cross-react with related polypeptide molecules. Antibodies and A β binding proteins herein are determined to be specifically reactive if they bind the target epitope with an affinity constant in the range of about 10^5 l/mole to 10^{12} l/mole, generally about 10^6 to 10^8 l/mole. In one embodiment, an antibody or A β binding protein is determined to be specifically reactive if it binds the target epitope with an affinity constant of at least about 10^5 l/mol, or at least about 10^6 l/mol. In a particular embodiment, an antibody or A β binding protein is determined to be specifically reactive if it binds the target epitope with an affinity constant of at least about 2×10^6 l/mol, or at least about 3×10^6 l/mol, or at least about 4×10^6 l/mol. The binding affinity of an antibody and an A β binding protein can be readily determined by one of skill in the art (Scatchard (1949) *Ann. N.Y. Acad. Sci.* 51: 660-672).

Selectivity of an antibody and an A β binding protein can refer to the degree of recognition of an antibody or A β binding protein for an antigen relative to other, particularly related, peptides or proteins. Selectivity or selectively reactive is considered a measure of the functional ability of an antibody to discriminate between the target antigen and other, chemically similar structures. In one aspect, selectivity of an antibody for a particular antigen relative to another peptide or protein can be determined by comparing the binding affinities of the antibody for the antigen and the other peptide. If the binding affinity (*e.g.*, as represented by an affinity constant) for the antigen is, for example, 1000-fold higher than that for the other peptide, the antibody can be said to be 1000-fold more selective or selectively reactive, for the antigen relative to the other

- 49 -

peptide.

As used herein, "bind preferentially" refers to the affinity of an A β binding protein, such as an A β antibody, for one antigen (such as an A β peptide or form) relative to another. For example, an A β binding protein can preferentially bind one A β form
5 relative to another A β form, such as preferentially binding low molecular weight forms of A β relative to high molecular weight forms of A β . In one embodiment, an A β binding protein binds preferentially to a particular A β form relative to another A β form if the A β binding protein binds the particular A β form with at least 2-fold higher affinity as compared with binding to the other A β form. In another embodiment, an A β binding
10 protein binds preferentially to a particular A β form relative to another A β form if the A β binding protein binds the particular A β form with at least 5-fold, 10-fold or more, including 20-fold and 100-fold higher affinity as compared with binding to the other A β form. In another embodiment, an A β binding protein binds preferentially to a particular A β peptide or form relative to another A β peptide or form if the binding of the A β
15 binding protein to the particular A β peptide or form can be detected in an immuno assay, such as western blot or ELISA assay, but the binding of the A β binding protein to another A β peptide or form is substantially less in the same or a similar assay. As used herein, "modulation" with reference to A β levels refers to any alteration or adjustment in cellular and/or extracellular or secreted A β , including, but not limited to, alteration of A β
20 concentration in the cytoplasm, cellular membranes, extracellular medium and/or intracellular organelles, *e.g.*, endoplasmic reticulum, endosome and lysosome, and any alteration of the production, clearance, and/or degradation of A β .

As used herein, "agent that modulates A β levels" refers to any substance that can modulate A β levels. Examples of agents include, but are not limited to, small organic
25 molecules, amino acids, peptides, polypeptides, nucleotides, nucleic acids, polynucleotides, carbohydrates, lipids, lipoproteins, glycoproteins, saccharides, fatty acids, steroids, purines, pyrimidines, derivatives, structural analogs or combinations thereof. Agents may be obtained from a wide variety of sources including libraries of synthetic or natural compounds. Libraries of natural compounds in the form of bacterial,
30 fungal, plant and animal extracts are available or readily produced. Additionally, natural

- 50 -

or synthetically produced libraries and compounds are readily modified through conventional chemical, physical and biochemical means, and may be used to produce combinatorial libraries. Known pharmacological agents may be subjected to directed or random chemical modifications, such as acylation, alkylation, esterification,
5 acidification, etc. to produce structural analogs.

As used herein, "test agent", in the context of methods for identifying agents that modulate A β levels, refers to any substance that is being evaluated as a possible agent that modulates A β levels.

As used herein, "amelioration" refers to an improvement in a disease or condition
10 or at least a partial relief of symptoms associated with a disease or condition.

As used herein, "substantially unchanged" or "without substantially altering or affecting" and variations thereof are used with reference to a particular composition, activity and/or process that is not a target for modulation. These expressions refer to the state (which includes amount or level) of the non-target composition, activity and/or
15 process under specified differing conditions. A composition may be, for example, a particular protein or peptide, such as an A β peptide, or a fragment or peptide generated by cleavage of a protein, such as a presenilin substrate. An activity or process may be, for example, the cleavage or processing of a protein such as a presenilin substrate. Differing conditions include any physical, chemical, environmental or other conditions in
20 which the composition, activity and/or process occurs. For example, differing conditions can be in the presence and absence of a test agent or agent that modulates a target composition, activity or process. A non-target composition, activity and/or process is substantially unchanged or is not substantially altered or affected if any variation in the composition, activity and/or process that occurs under specified differing conditions is an
25 acceptable variation. Those of skill in the art can identify acceptable variation. For example, acceptable variation generally can be any alteration in the composition, activity and/or process (including, *e.g.*, increase or decrease in the amount or level) that is less than or relatively minimal in comparison to the variation in a target composition, process or activity under the specified differing conditions, or that is not associated with an
30 undesired effect. An undesired effect can be, for example, an adverse effect on a

- 51 -

biological composition, cell, tissue, system or organism including or containing the cell or composition. Undesired effects include, for example, deleterious alterations in any aspect of cell function, decreased cell viability and cell death. Acceptable variation can also be any alteration in the composition, activity and/or process that is inconsequential (or without significant consequence) to an overall or ultimate downstream function in which the composition, activity and/or process is involved. Thus, in a particular example of a peptide that is not a target for modulation, substantially unchanged with respect to the levels of such a non-target peptide in the presence and absence of an agent being tested as a possible modulator of a target peptide means that there is no change, or an acceptable variation, in the level of the non-target peptide in the presence of the agent compared to in the absence of the agent. Acceptable variation in a non-target composition, activity and/or process (including levels or amounts) may be different for different compositions, activities and processes, and in the context of different sets of specified differing conditions. In some particular instances, acceptable variation can range from equal to or less than about 40, 30, 20, or 10% variation when compared under differing conditions, *e.g.*, in the presence and absence of a test agent. It should be understood that this definition of "substantially unchanged" or "without substantially altering or affecting" applies and is used with reference to a composition, activity and/or process that is not a target for modulation. In contrast, any variation (and particularly a statistically significant variation) in a composition, activity and/or process that is a target for modulation in the presence and absence of a test agent can be a sufficient modulation.

As used herein, "avidity" refers to the functional affinity or combining strength of an antibody with its antigen and is related to both the affinity of the reaction between the epitopes and paratopes, and the valences or recognition sites of the antibody and antigen.

25

As used herein, "selective modulation of A β levels" refers to the modulation of the levels of one or more forms of A β , wherein one or more other specified compositions or specified activities, processes or mechanisms are substantially unchanged, or without substantially altering or affecting one or more other specified compositions or specified activities, processes or mechanisms. For example, selective modulation of an A β peptide

30

- 52 -

can be relative to one or more other related polypeptide molecules (*e.g.*, other A β peptides) in which the level of a particular A β peptide is modulated without substantially altering the levels of one or more other A β peptides. In another example, selective modulation of an A β peptide can be relative to the processing of a presenilin substrate
5 other than APP, in which A β levels are modulated without substantially altering the cleavage of the presenilin substrate that is other than APP.

As used herein, "related peptide molecules" refers to any peptide molecules with chemically similar structures, any peptides molecules that undergo similar processing by the same or similar enzymes, any peptide molecules derived from the same or similar
10 precursor peptide molecule, and/or any peptide molecules that have the same or similar activities and/or functions.

As used herein, "treatment" means any manner in which the symptoms of a conditions, disorder or disease are ameliorated or otherwise beneficially altered. Treatment also encompasses any pharmaceutical use of the compositions herein.

15 As used herein, a "combination" refers to any association between two or among more items.

As used herein, an "agent identified by the screening methods provided herein for identifying candidate agents for the treatment and/or prevention of a disease or disorder" refers to any compound that is a candidate for use as a therapeutic or as lead compound
20 for design of a therapeutic. Such compounds can be small molecules, including small organic molecules, peptides, peptide mimetics, antisense molecules or dsRNA, such as RNAi, antibodies, fragments of antibodies, recombinant antibodies and other such compound which can serve as drug candidate or lead compound.

As used herein, a "peptidomimetic" is a compound that mimics the conformation
25 and certain stereochemical features of the biologically active form of a particular peptide. In general, peptidomimetics are designed to mimic certain desirable properties of a compound, but not the undesirable properties, such as flexibility, that lead to a loss of a biologically active conformation and bond breakdown. Peptidomimetics may be prepared from biologically active compounds by replacing certain groups or bonds that
30 contribute to the undesirable properties with bioisosteres. Bioisosteres are known to

- 53 -

those of skill in the art. For example the methylene bioisostere CH₂S has been used as an amide replacement in enkephalin analogs (see, e.g., Spatola (1983) pp. 267-357 in Chemistry and Biochemistry of Amino Acids, Peptides, and Proteins, Weistein, Ed. volume 7, Marcel Dekker, New York). Morphine, which can be administered orally, is a
5 compound that is a peptidomimetic of the peptide endorphin. For purposes herein, cyclic peptides are included among peptidomimetics.

As used herein, "production by recombinant means by using recombinant DNA methods" means the use of the well known methods of molecular biology for expressing proteins encoded by cloned DNA.

10 As used herein, "heterologous" or "foreign" with reference to nucleic acids, cDNA, DNA and RNA are used interchangeably and refer to nucleic acid, DNA or RNA that does not occur naturally as part of the genome in which it is present or which is found in a location(s) or in an amount in the genome that differs from that in which it occurs in nature. It can be nucleic acid that has been exogenously introduced into the
15 cell. Thus, heterologous nucleic acid is nucleic acid not normally found in the host genome in an identical context. Examples of heterologous nucleic acids include, but are not limited to, DNA that encodes a gene product or gene product(s) of interest, introduced, for example, for purposes of gene therapy or for production of an encoded protein. Other examples of heterologous DNA include, but are not limited to, DNA that
20 encodes a selectable marker, DNA that encodes therapeutically effective substances, such as anti-cancer agents, enzymes and hormones, and DNA that encodes other types of proteins, such as antibodies.

As used herein, "expression" refers to the process by which nucleic acid, e.g., DNA, is transcribed into mRNA and translated into peptides, polypeptides, or proteins.
25 If the nucleic acid is derived from genomic DNA, expression may, if an appropriate eukaryotic host cell or organism is selected, include splicing of the mRNA.

As used herein, "vector" or "plasmid" refers to discrete elements that are used to introduce heterologous nucleic acids into cells. Typically, vectors are used to transfer heterologous nucleic acids into cells for either expression of the heterologous nucleic
30 acid or for replication of the heterologous nucleic acid. Selection and use of such vectors

- 54 -

and plasmids are well within the level of skill of the art.

As used herein, "transformation" or "transfection" refers to the process by which nucleic acids are introduced into cells. Transfection refers to the taking up of exogenous nucleic acid, e.g., an expression vector, by a host cell whether or not any coding
5 sequences are in fact expressed. Numerous methods of transfection are known to the ordinarily skilled artisan. Successful transfection is generally recognized by detection of the presence of the heterologous nucleic acid within the transfected cell, such as, for example, any visualization of the heterologous nucleic acid or any indication of the operation of a vector within the host cell.

10 As used herein, "injection" refers to the microinjection (use of a small syringe) of nucleic acid into a cell.

As used herein, the amino acids, which occur in the various amino acid sequences appearing herein, are identified according to their well-known, three-letter or one-letter abbreviations (see Table 1). The nucleotides, which occur in the various DNA
15 fragments, are designated with the standard single-letter designations used routinely in the art.

As used herein, "amino acid residue" refers to an amino acid formed upon chemical digestion (hydrolysis) of a polypeptide at its peptide linkages. The amino acid residues described herein are preferably in the "L" isomeric form. However, residues in
20 the "D" isomeric form can be substituted for any L-amino acid residue, as long as the desired functional property is retained by the polypeptide. NH₂ refers to the free amino group present at the amino terminus of a polypeptide. COOH refers to the free carboxy group present at the carboxyl terminus of a polypeptide. In keeping with standard polypeptide nomenclature described in *J. Biol. Chem.*, 243:3552-59 (1969) and adopted
25 at 37 C.F.R. § 1.821 - 1.822, abbreviations for amino acid residues are shown in Table 1:

Table 1 - Table of Correspondence

SYMBOL		
1-Letter	3-Letter	AMINO ACID
Y	Tyr	tyrosine
G	Gly	glycine
F	Phe	phenylalanine
M	Met	methionine
A	Ala	alanine
S	Ser	serine
I	Ile	isoleucine
L	Leu	leucine
T	Thr	threonine
V	Val	valine
P	Pro	proline
K	Lys	lysine
H	His	histidine
Q	Gln	glutamine
E	Glu	glutamic acid
Z	Glx	Glu and/or Gln
W	Trp	tryptophan
R	Arg	arginine
D	Asp	aspartic acid
N	Asn	asparagine
B	Asx	Asn and/or Asp
C	Cys	cysteine
X	Xaa	Unknown or other

- 56 -

It should be noted that all amino acid residue sequences represented herein by formulae have a left to right orientation in the conventional direction of amino-terminus to carboxyl-terminus. In addition, the phrase "amino acid residue" is broadly defined to include the amino acids listed in the Table of Correspondence and modified and unusual amino acids, such as those referred to in 37 C.F.R. § § 1.821-1.822, and incorporated herein by reference. Furthermore, it should be noted that a dash at the beginning or end of an amino acid residue sequence indicates a peptide bond to a further sequence of one or more amino acid residues or to an amino-terminal group such as NH₂ or to a carboxyl-terminal group such as COOH.

In a peptide or protein, suitable conservative substitutions of amino acids are known to those of skill in this art and may be made generally without altering the biological activity of the resulting molecule. Those of skill in this art recognize that, in general, single amino acid substitutions in non-essential regions of a polypeptide do not substantially alter biological activity (see, e.g., Watson et al. *Molecular Biology of the Gene*, 4th Edition, 1987, The Benjamin/Cummings Pub. co., p.224).

Such substitutions may be made in accordance with those set forth in TABLE 2 as follows:

Table 2

Original residue	Conservative substitution
Ala (A)	Gly; Ser
Arg (R)	Lys
Asn (N)	Gln; His
Cys (C)	Ser
Gln (Q)	Asn
Glu (E)	Asp
Gly (G)	Ala; Pro
His (H)	Asn; Gln
Ile (I)	Leu; Val
Leu (L)	Ile; Val
Lys (K)	Arg; Gln; Glu

- 57 -

Met (M)	Leu; Tyr; Ile
Phe (F)	Met; Leu; Tyr
Ser (S)	Thr
Thr (T)	Ser
Trp (W)	Tyr
Tyr (Y)	Trp; Phe
Val (V)	Ile; Leu

Other substitutions are also permissible and may be determined empirically or in accord with known conservative substitutions.

As used herein, all assays and procedures, such as hybridization reactions and
 5 antibody-antigen reactions, unless otherwise specified, are conducted under conditions recognized by those of skill in the art as standard conditions.

As used herein: "stringency of hybridization" in determining percentage mismatch is as follows:

- 1) high stringency: 0.1 x SSPE, 0.1% SDS, 65°C
- 10 2) medium stringency: 0.2 x SSPE, 0.1% SDS, 50°C
- 3) low stringency: 1.0 x SSPE, 0.1% SDS, 50°C

Those of skill in this art know that the washing step selects for stable hybrids and also know the ingredients of SSPE (see, e.g., Sambrook, E.F. Fritsch, T. Maniatis, in: Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory Press (1989),
 15 vol. 3, p. B.13, see, also, numerous catalogs that describe commonly used laboratory solutions). SSPE is pH 7.4 phosphate- buffered 0.18 NaCl. Further, those of skill in the art recognize that the stability of hybrids is determined by T_m , which is a function of the sodium ion concentration and temperature ($T_m = 81.5^\circ \text{C} - 16.6(\log_{10}[\text{Na}^+]) + 0.41(\%G+C) - 600/l$), so that the only parameters in the wash conditions critical to
 20 hybrid stability are sodium ion concentration in the SSPE (or SSC) and temperature.

It is understood that equivalent stringencies can be achieved using alternative buffers, salts and temperatures. By way of example and not limitation, procedures using conditions of low stringency are as follows (see also Shilo and Weinberg, *Proc. Natl. Acad. Sci. USA*, 78:6789-6792 (1981)): Filters containing DNA are pretreated for 6

- 58 -

hours at 40°C in a solution containing 35% formamide, 5X SSC, 50 mM Tris-HCl (pH 7.5), 5 mM EDTA, 0.1% PVP, 0.1% Ficoll®, 1% BSA, and 500 µg/ml denatured salmon sperm DNA (10X SSC is 1.5 M sodium chloride, and 0.15 M sodium citrate, adjusted to a pH of 7).

5 Hybridizations are carried out in the same solution with the following modifications: 0.02% PVP, 0.02% Ficoll, 0.2% BSA, 100 µg/ml salmon sperm DNA, 10% (wt/vol) dextran sulfate, and 5-20 X 10⁶ cpm ³²P-labeled probe is used. Filters are incubated in hybridization mixture for 18-20 hours at 40°C, and then washed for 1.5 hours at 55°C in a solution containing 2X SSC, 25 mM Tris-HCl (pH 7.4), mM
10 EDTA, and 0.1% SDS. The wash solution is replaced with fresh solution and incubated an additional 1.5 hours at 60°C. Filters are blotted dry and exposed for autoradiography. If necessary, filters are washed for a third time at 65-68°C and reexposed to film. Other conditions of low stringency which can be used are well known in the art (*e.g.*, as employed for cross-species hybridizations).

15 By way of example and not way of limitation, procedures using conditions of moderate stringency is provided. For example, but not limited to, procedures using such conditions of moderate stringency are as follows: Filters containing DNA are pretreated for 6 hours at 55°C in a solution containing 6X SSC, 5X Denhart's solution, 0.5% SDS and 100 µg/ml denatured salmon sperm DNA. Hybridizations are carried out in the same
20 solution and 5-20 X 10⁶ cpm ³²P-labeled probe is used. Filters are incubated in hybridization mixture for 18-20 hours at 55°C, and then washed twice for 30 minutes at 60°C in a solution containing 1X SSC and 0.1% SDS. Filters are blotted dry and exposed for autoradiography. Other conditions of moderate stringency which can be used are well-known in the art. Washing of filters is done at 37°C for 1 hour in a
25 solution containing 2X SSC, 0.1% SDS.

 By way of example and not way of limitation, procedures using conditions of high stringency are as follows: Prehybridization of filters containing DNA is carried out for 8 hours to overnight at 65°C in buffer composed of 6X SSC, 50 mM Tris-HCl (pH 7.5), 1 mM EDTA, 0.02% PVP, 0.02% Ficoll, 0.02% BSA, and 500 µg/ml denatured
30 salmon sperm DNA. Filters are hybridized for 48 hours at 65°C in prehybridization

- 59 -

mixture containing 100 $\mu\text{g/ml}$ denatured salmon sperm DNA and $5\text{--}20 \times 10^6$ cpm of ^{32}P -labeled probe. Washing of filters is done at 37°C for 1 hour in a solution containing 2X SSC, 0.01% PVP, 0.01% Ficoll, and 0.01% BSA. This is followed by a wash in 0.1X SSC at 50°C for 45 minutes before autoradiography. Other conditions of high stringency
5 which can be used are well known in the art.

As used herein, "substantially identical to a product" means sufficiently similar so that the property of interest is sufficiently unchanged so that the substantially identical product can be used in place of the product.

As used herein, "isolated" when used with reference to a composition such as an
10 antibody or portion or fragment thereof or to a protein means that such composition is in a state that is not identical to that as it may occur in nature, if it occurs in nature. Such an isolated composition typically has been manipulated or altered from its naturally occurring state in some way by the hand of man.

As used herein, "substantially pure" means sufficiently homogeneous to appear
15 free of readily detectable impurities as determined by standard methods of analysis, such as thin layer chromatography (TLC), gel electrophoresis and high performance liquid chromatography (HPLC), used by those of skill in the art to assess such purity, or sufficiently pure such that further purification would not detectably alter the physical and chemical properties, such as enzymatic and biological activities, of the substance.
20 Methods for purification of the compounds to produce substantially chemically pure compounds are known to those of skill in the art. A substantially chemically pure compound can, however, be a mixture of stereoisomers or isomers. In such instances, further purification might increase the specific activity of the compound.

As used herein, "target cell" refers to a cell that contains a target molecule of
25 interest, for example, an APP and/or A β peptide(s).

As used herein, "test substance" refers to a chemically defined compound (*e.g.*, organic molecules, inorganic molecules, organic/inorganic molecules, proteins, peptides, nucleic acids, oligonucleotides, lipids, polysaccharides, saccharides, or hybrids among these molecules such as glycoproteins) or mixtures of compounds (*e.g.*, a library of test
30 compounds, natural extracts or culture supernatants) whose effect on a target of interest,

- 60 -

e.g., A β peptides and/or levels thereof in a sample, is sought to be determined by, for example, methods and assays provided herein.

As used herein, the terms "a therapeutic agent," "therapeutic regimen," "radioprotectant," "chemotherapeutic" mean conventional drugs and drug therapies, including antibodies, which are known to those skilled in the art. Radiotherapeutic agents are well known in the art.

As used herein, by "homologous" (with respect to nucleic acid and/or amino acid sequences) means about greater than or equal to 25% sequence homology, typically greater than or equal to 25%, 40%, 60%, 70%, 80%, 85%, 90% or 95% sequence homology; the precise percentage can be specified if necessary. For purposes herein the terms "homology" and "identity" are often used interchangeably, unless otherwise indicated. In general, for determination of the percentage homology or identity, sequences are aligned so that the highest order match is obtained (see, e.g.: *Computational Molecular Biology*, Lesk, A.M., ed., Oxford University Press, New York, 1988; *Biocomputing: Informatics and Genome Projects*, Smith, D.W., ed., Academic Press, New York, 1993; *Computer Analysis of Sequence Data, Part I*, Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994; *Sequence Analysis in Molecular Biology*, von Heinje, G., Academic Press, 1987; and *Sequence Analysis Primer*, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991; Carillo *et al.* (1988) *SIAM J Applied Math* 48:1073). By sequence homology, the number of conserved amino acids are determined by standard alignment algorithms programs, and are used with default gap penalties established by each supplier. Substantially homologous nucleic acid molecules would hybridize typically at moderate stringency or at high stringency all along the length of the nucleic acid of interest. Also contemplated are nucleic acid molecules that contain degenerate codons in place of codons in the hybridizing nucleic acid molecule.

Whether any two nucleic acid molecules have nucleotide sequences that are at least 60%, 70%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% "identical" or "homologous" can be determined using known computer algorithms such as the "FAST A" program, using for example, the default parameters as in Pearson *et al.* (1988) *Proc.*

- 61 -

Natl. Acad. Sci. USA 85:2444 (other programs include the GCG program package (Devereux, J., *et al.*, *Nucleic Acids Research* 12(I):387 (1984)), BLASTP, BLASTN, FASTA (Atschul, S.F., *et al.*, *J Molec Biol* 215:403 (1990); Guide to Huge Computers, Martin J. Bishop, ed., Academic Press, San Diego, 1994, and Carillo *et al.* (1988) *SIAM J Applied Math* 48:1073). For example, the BLAST function of the National Center for Biotechnology Information database can be used to determine identity. Other commercially or publicly available programs include, DNASTar "MegAlign" program (Madison, WI) and the University of Wisconsin Genetics Computer Group (UWG) "Gap" program (Madison WI). Percent homology or identity of proteins and/or nucleic acid molecules can be determined, for example, by comparing sequence information using a GAP computer program (*e.g.*, Needleman *et al.* (1970) *J. Mol. Biol.* 48:443, as revised by Smith and Waterman ((1981) *Adv. Appl. Math.* 2:482). Briefly, the GAP program defines similarity as the number of aligned symbols (*i.e.*, nucleotides or amino acids) which are similar, divided by the total number of symbols in the shorter of the two sequences. Default parameters for the GAP program can include: (1) a unary comparison matrix (containing a value of 1 for identities and 0 for non-identities) and the weighted comparison matrix of Gribskov *et al.* (1986) *Nucl. Acids Res.* 14:6745, as described by Schwartz and Dayhoff, eds., *ATLAS OF PROTEIN SEQUENCE AND STRUCTURE*, National Biomedical Research Foundation, pp. 353-358 (1979); (2) a penalty of 3.0 for each gap and an additional 0.10 penalty for each symbol in each gap; and (3) no penalty for end gaps.

Therefore, as used herein, the term "identity" or "homology" represents a comparison between a test and a reference polypeptide or polynucleotide. As used herein, the term at least "90% identical to" refers to percent identities from 90 to 99.99 relative to the reference nucleic acid or amino acid sequences. Identity at a level of 90% or more is indicative of the fact that, assuming for exemplification purposes a test and reference polypeptide length of 100 amino acids are compared. No more than 10% (*i.e.*, 10 out of 100) amino acids in the test polypeptide differs from that of the reference polypeptides. Similar comparisons can be made between a test and reference polynucleotides. Such differences can be represented as point mutations randomly

- 62 -

distributed over the entire length of an amino acid sequence or they can be clustered in one or more locations of varying length up to the maximum allowable, e.g. 10/100 amino acid difference (approximately 90% identity). Differences are defined as nucleic acid or amino acid substitutions, or deletions. At the level of homologies or identities above
5 about 85-90%, the result should be independent of the program and gap parameters set; such high levels of identity can be assessed readily, often without relying on software.

As used herein, "primer" refers to an oligonucleotide containing two or more deoxyribonucleotides or ribonucleotides, generally more than three, from which synthesis of a primer extension product can be initiated. Experimental conditions conducive to
10 synthesis include the presence of nucleoside triphosphates and an agent for polymerization and extension, such as DNA polymerase, and a suitable buffer, temperature and pH.

As used herein, "animals" include any animal, such as, but are not limited to, goats, cows, deer, sheep, rodents, pigs and humans. Non-human animals, exclude
15 humans as the contemplated animal.

As used herein, the term "subject" is used interchangeably with the term "individual" and includes mammals, such as humans.

B. Pathogenesis of Alzheimer's Disease

Neuropathologically, AD is characterized by massive neuronal cell loss in certain
20 brain areas, and by the deposition of proteinaceous material in the brains of AD patients. These deposits are the neurofibrillary tangles and the β -amyloid plaques. The major protein component of the β -amyloid plaque is the $A\beta$ peptide which is derived from processing of amyloid precursor protein (APP). Increased accumulation of $A\beta$ peptide has been postulated to be a causal factor in the pathogenesis of AD. Supportive evidence
25 for the causal role of $A\beta$ in AD can be found in patients with Down's syndrome, who often develop AD-like symptoms and pathology after age 40. Down's syndrome patients produce elevated APP presumably due to an additional copy of chromosome 21 and exhibit AD-like amyloid plaques prior to the onset of other AD symptoms, suggesting that increased amyloid accumulation is an initial event (Giaccone G. *et al.*, (1989)
30 *Neurosci Lett* 97:232-8). Additional evidence implicating accumulation of $A\beta$ peptides

- 63 -

in AD comes from various recently identified mutations accounting for certain types of inherited AD. For example, alterations in APP processing have been linked to a subset of familial AD patients (FAD) with autosomal dominant mutations in APP (Goate, A. et al., (1991) *Nature* 349:704-6; Citron, M. et al., (1992) 360:672-4), presenilin 1
5 (Sherrington, R. et al., (1995) *Nature* 375:754-60), and presenilin 2 (Levy-Lehad, E., et al., (1995) *Science* 269:970-3). FAD individuals comprise 10% of all AD cases and generally exhibit symptoms of the disease much earlier than sporadic AD patients. For example, a double mutation of amino acids 670 and 671 of APP from Lys-Met to Asn-Lys, respectively, immediately upstream of the β -cleavage site of $A\beta$ ("Swedish"
10 mutation or APP_{ANL}) results in a 5-8-fold increase in the formation of $A\beta$ by cells (Citron, M. et al., (1992) 360:672-4). The fact that such alterations are sufficient to cause AD-like pathology is supported by studies which show that transgenic mice overexpressing APP_{ANL} (Hsiao, K., et al., (1996) *Science* 274:99-102) produce higher levels of $A\beta$ prior to the exhibition of other AD pathological features such as abnormal
15 phosphorylation of cytoskeletal tau, microgliosis, reactive astrocytosis, reduced levels of synaptic marker proteins and memory deficits.

1. $A\beta$ production

$A\beta$ peptides are derived from processing of an amyloid precursor protein (APP). Although there are several isoforms of APP, forms that contain a single-transmembrane
20 protein have an approximately 590-680 amino acid long extracellular amino-terminal domain and an approximately 55 amino acid cytoplasmic tail which contains intracellular trafficking signals. Within APP, the $A\beta$ peptide sequence is located partially on the extracellular side of the membrane and extends partially into the transmembrane region. Positions 29-42 on the $A\beta$ peptide lie entirely within the putative transmembrane region
25 and are hydrophobic in nature (Miller *et al.* (1993) *Arch. Biochem. Biophys* 301:41-52). mRNA generated from the APP gene on chromosome 21 undergoes alternative splicing to yield about 10 possible isoforms, three of which (the 695, 751, and 770 amino acid isoforms; see SEQ ID NOs: 30, 28 and 2, respectively, for exemplary amino acid sequences) predominate in the brain. APP₆₉₅ is the shortest of the three isoforms and is
30 produced mainly in neurons. Alternatively, APP₇₅₁, which contains a Kunitz-protease

- 64 -

inhibitor (KPI) domain, and APP₇₇₀, which contains both the KPI domain and an MRC-OX2 antigen domain, are found mostly in non-neuronal glial cells. All three isoforms share the same A β , transmembrane, and intracellular domains and are thus all potentially amyloidogenic.

5 APP is trafficked through the constitutive secretory pathway, where it undergoes post-translational processing including a variety of proteolytic cleavage events. APP can undergo proteolytic processing via two pathways: an amyloidogenic pathway and a non-amyloidogenic pathway. In the non-amyloidogenic pathway, cleavage of APP by α -secretase occurs within the A β domain releasing a large soluble N-terminal fragment
10 (sAPP α) for secretion and a non-amyloidogenic C-terminal fragment (C83) of about 10 kD. Because α -secretase cleaves within the A β domain, this cleavage precludes A β formation. Rather, the C-terminal fragment of APP generated by α -secretase cleavage is subsequently cleaved by γ -secretase within the predicted transmembrane domain to generate a 22-24 residue non-amyloidogenic peptide fragment termed p3. Alternatively,
15 in the amyloidogenic pathway, cleavage of APP by β -secretase (BACE) occurs at the beginning of the A β domain defining the amino terminus of the A β peptide. This cleavage generates a shorter soluble N-terminus, APP β , as well as an amyloidogenic C-terminal fragment (C99). Further cleavage of this C-terminal fragment by γ -secretase, a presenilin-dependent enzyme, generates A β .

20 Cleavage by distinct γ -secretase activities and/or multiple γ -secretases results in C-terminal heterogeneity of A β , generating fragments of various lengths. For example, A β ₄₀ and A β ₄₂, which contain 40 and 42 amino acids, respectively (see, e.g., SEQ ID NO: 4; amino acids 1-40 and 1-42), are thought to be cleaved by a cysteine protease and a serine protease, respectively (Figueiredo-Pereira *et al.* (1999) *J. Neurochem.*
25 72(4):1417-22). Thus, selective modulation of the production of a particular form of A β should be possible by targeting appropriate enzymes.

The predominant forms of A β found in plaques are the A β ₄₀ and A β ₄₂ variants. A β ₄₂ accumulates primarily intracellularly, representing only 5-15% of the total A β secreted by most cell lines (Wang, *et al.*, (2001) *Neurobiology of Aging* 23:213-223).
30 Published immunohistochemical studies have demonstrated that in brains of individuals

- 65 -

harboring FAD-linked mutations in APP (Val to Ile at codon 717), A β 42 is deposited early and selectively in the cerebral cortex. This holds true in numerous studies with transgenic mice and in FAD patients harboring mutations in presenilin genes known to increase A β 42 formation (relative to A β 40). In the AD cerebral cortex, virtually all AD

5 plaques are A β 42 immunopositive while only approximately one third are A β 40 immunopositive. In fact, diffuse amyloid plaques, representing the earliest stage of amyloid deposition, are almost exclusively composed of A β 42 (Iwatsubo *et al.* (1994) *Neuron* 13: 45053; Borchelt *et al.* (1997) *Neuron* 19: 939). In vitro experiments have demonstrated that A β 42 polymerizes faster than A β 40, suggesting that the carboxy

10 terminus of A β determines the aggregation potential, and therefore, is one of the critical determinants for the rate of amyloid fibril formation (Parvathy, *et al.*, (2001) *Arch Neurol.* 58: 2025-2032). A β 42 has also been shown to dramatically enhance precipitation of A β 40 in vitro. Therefore, the A β 42 species of amyloid peptide is a

15 primary target in the development of therapeutics for the treatment of neurodegenerative disease characterized by A β plaque formation. A β 42 accumulation predominantly affects neurons in the cerebral cortex and hippocampus of AD brains prior to the appearance of amyloid plaques. Neurons burdened with excessive A β 42 can lose function and eventually undergo lysis, resulting in local dispersal of their cytoplasmic contents.

Production of A β can occur at several distinct locations along the secretory

20 pathway. APP produced in the endoplasmic reticulum (ER) transits to the Golgi, where it is post-translationally modified via N- and O-linked glycosylation and tyrosine sulfation before vesicular transport to the cell surface. Cell surface APP is then reinternalized via endocytosis into the endosomal/lysosomal system where it may be degraded. Cleavage of APP to form A β can occur in at least three sites along this

25 pathway. The endosomal-lysosomal system may contribute minor amounts of secreted A β , particularly in non-neuronal cells. The trans-Golgi network (TGN) is the major site of intracellular A β 40 production in neurons and in non-neuronal cells transfected with mutant APP. In addition, either the TGN or post-Golgi vesicles are responsible for the production of secreted A β in neurons. Finally, the ER is a site for the production of

30 A β 42. A β 42 produced in the ER is found in an intracellular stable insoluble pool. The

- 66 -

proteosome may aid in the degradation of these ER-generated APP fragments (Skovronsky (2000) *Biochemistry* 39(4):810-7). Due to the organelle-specific differences in the generation and clearance/degradation of A β peptides, it is possible to selectively modulate the production, clearance and/or degradation of a particular form of A β by targeting appropriate γ -secretases and/or degradative enzymes.

Presenilins, multitransmembrane proteins localized predominantly to the ER and Golgi, play a crucial role in APP processing. Presenilin-1 (PS-1) was first identified as an early onset gene in Alzheimer's disease and is believed to be a critical component of the enzyme complex which cleaves the amyloid precursor protein (APP) at the γ -secretase site to produce A β . Over 40 dominant point mutations in PS-1 (chromosome 14) and PS-2 (chromosome 1) as well as one splice site mutation in PS-1 have been associated with familial AD (FAD) phenotypes (see, e.g., Van Gassen *et al.* (2000) *Neurobiol. Dis.* 7:135-151; Hardy (1997) *Trends Neurosci.* 20:154-159; and Cruts and Van Broeckhoven (1998) *Ann. Med.* 30:560-565). Thus, presenilins are involved in the carboxy-terminal cleavage of APP in both normal and pathological states. Involvement of presenilin has also been shown in the cleavage of additional membrane proteins such as Notch, Erb-B4 (Lee *et al.* 2002, *J. Biol. Chem.* 277(8):6318-23), and E-cadherin (Marambaud *et al.* 2002, *EMBO J.* 21(8):1948-56). Presenilins may play a general role in intramembrane cleavage and, thus, may likely have additional substrates yet to be reported.

2. A β degradation/clearance

The accumulation of A β 42 in the brain clearly depends on the production levels of the amyloid peptide, however numerous other factors also contribute significantly to brain A β 42 levels. Some of these factors are A β 42 proteolytic degradation, receptor-mediated clearance, non-receptor-mediated clearance, and/or aggregation/fibrillogenesis. Therefore, defects in pathways for A β degradation and clearance could underlie some or many cases of familial and sporadic AD as well as other diseases and disorders characterized by misregulation of A β . Understanding how A β degradation and clearance is regulated in the cerebral cortex has implications for both the pathogenesis and the treatment of such diseases and disorders. Agents that affect any of these

- 67 -

pathways/mechanisms can be useful as therapeutic drugs.

Metabolic labeling studies in living mice show that newly generated A β is very rapidly turned over in the brain (Savage *et al.*, (1998), *J. Neurosci* 18:1743-1752), suggesting that A β -degradation proteases help regulate its levels. There are numerous proteases in the brain that could potentially participate in A β turnover, and there is evidence that several enzymes may contribute to the degradation of A β peptides in brain tissue including insulin-degrading enzyme (IDE), neprilysin, plasmin, uPA/tPA, endothelin converting enzyme-1, and matrix metalloproteinase-9 (Selkoe J. (2001) *Neuron* 32:177-180). IDE has been shown to degrade insulin, glucagon, atrial natriuretic peptide, calcitonin, TGF- α , and amylin, among other small peptides of diverse sequence. IDE is believed to have little dependence on sequence specificity but recognizes a conformation that is prone to conversion to a β -pleated sheet structure. Such a property is concurrent with its propensity to degrade several peptides that undergo concentration dependent formation of amyloid fibrils (e.g., insulin, ANF, amylin, calcitonin, and A β). It is believed that the motif recognized by IDE is not the β -pleated sheet region per se but a conformation of the monomer in a pre-amyloid state. IDE occurs principally in a soluble form in the cytoplasm. However, a form of IDE can be labeled on the cell surface, including in neurons, and is also present on intracellular membranes (Vekrellis *et al.* (2000) *J. Neurosci.* 20: 1657-1665). The existence of a membrane-anchored form of the protease suggests that it could help regulate insulin signaling at the plasma membrane and could also participate in the degradation of both soluble and membrane-associated forms of A β .

Neprilysin is a member of the neutral endopeptidase family of membrane-anchored proteases found on the cell surface. Neprilysin has been implicated in the degradation of A β peptides (Iwata *et al.*, (2000) *Nat. Med.* 6:143-150; Carson and Turner, (2002) *J. Neurochem* 81(1): 1-8), mediating the degradation of predominantly insoluble forms of A β . In addition, it has been shown that steady state levels of endogenous A β are elevated in the brains of young neprilysin-deficient mice (Iwata *et al.* (2001) *Science* 292: 1550-1552). The rise, while highly significant, was not large, and plaque formation was not observed. Thus, it is believed that other proteases, including

- 68 -

additional members of the neutral endopeptidase family, may function to degrade A β .

The plasmin proteolytic cascade, known to be crucial for fibrinolysis and cell migration, has been implicated in A β clearance as well. In this cascade, either of two activators of plasmin, tissue-type plasminogen activator (tPA) or urokinase-type plasminogen activator (uPA), can be post-translationally activated by binding to fibrin and other substrates. In vitro studies have suggested that A β aggregates can substitute for fibrin aggregates in activating tPA. In the nervous system, plasminogen, tPA and uPA are expressed in neurons, and tPA is also synthesized by microglia. In vitro assays have indicated that pure plasmin can proteolyze monomeric A β and fibrillar A β at a considerably lower efficiency.

Another protease expressed in brain that has been evaluated for its ability to degrade A β is endothelin converting enzyme-1 (ECE-1) (Eckman *et al.* (2001) *J. Biol. Chem.* 276: 24540-24548). This integral membrane zinc metalloprotease, with its active site located in the lumen and extracellularly, can cleave the endothelin precursors and several other biologically active peptides, including bradykinin, substance P, and the oxidized insulin B chain. Cellular overexpression of ECE-1 leads to a marked reduction in the levels of naturally secreted A β 40 and A β 42 peptides in Chinese hamster ovary cells. The purified enzyme directly proteolyzed both synthetic peptides in vitro. Other purified proteases that have been reported to digest synthetic A β peptides under in vitro conditions include matrix metalloproteinase-9 and cathepsin D.

In addition, several cell surface receptors have been implicated in A β clearance, including the scavenger receptor A (Paresce *et al.*, (1996) *Neuron* 17:553-565), the receptor for advanced glycation endproducts (RAGE) (Yan *et al.*, (1996) *Nature* 382: 685-691), and the low-density lipoprotein receptor-related protein-1 and -2 (LRP-1 and LRP-2) (Narita *et al.*, (1997) *J. Neurochem.* 69:1904-1911; Shibata *et al.*, (2000) *J. Clin. Invest.* 106(12): 1489-99; Kang *et al.*, (2000) *J. Clin. Invest.* 106(9): 1159-66; Ulery and Strickland, (2000) *J. Clin. Invest.* 106(15): 1077-9; Hammad *et al.*, (1997) *J. Biol. Chem.* 272(30): 18644-9). Scavenger receptor binding to A β has been shown to facilitate the uptake of A β by microglia. Microglia are immune system cells associated with Alzheimer's disease plaques containing A β . These cells facilitate phagocytosis of

- 69 -

amyloid fibrils into the endosomal/lysosomal system where they may subsequently be degraded by acid hydrolases in late endosomes and lysosomes (Selkoe (2001) *Neuron* 32: 177-180). The scavenger receptors expressed by microglia appear to play a significant role in this clearance process and, thus may be useful targets for the identification of agents that modulate A β levels.

Binding of A β to neuronal RAGE induces activation of nuclear factor κ B (NF- κ B), which drives expression of macrophage-colony stimulating factor (M-CSF). M-CSF signals microglia from distant sites, drawing them toward loci of neuronal perturbation and inducing cell activation, including increased proliferation, and enhanced expression of microglial scavenger receptors and apoE. Such activation may lead to increased clearance of A β through microglial phagocytic pathways.

LRP is a multifunctional receptor with four distinct ligand binding domains and at least 14 identified ligands, including apolipoprotein E (apoE), apoJ, α 2-macroglobulin (α 2M), and lactoferrin. LRP is involved in receptor-mediated endocytosis, directing ligands to degradation via the late endosome and lysosome. A β has been found to bind several LRP ligands including apoE (Holtzman, (2001) *J. Mol. Neurosci.* 17(2):147-55), apoJ (Hammad *et al.*, (1997) *J. Biol. Chem.* 272(30): 18644-9), and activated α 2M (α 2M*) (Qiu *et al.*, (1999) *J. Neurochem* 73(4):1393-8). Such ligand interactions, and specifically the binding of A β to α 2M*, are believed to facilitate A β clearance through an LRP-mediated endocytic pathway. Identification of agents which modulate LRP and/or components of LRP-mediated clearance pathways provides an attractive approach for therapeutic intervention.

The proteasome has also been implicated in the degradation of ER-generated APP fragments, specifically A β 42 (Skovronsky (2000) *Biochemistry* 39(4):810-7). General phagocytic mechanisms and up-regulation of genes in response to inflammatory stimuli are also reported to enhance A β clearance. In addition, metal chelators, such as clioquinol (Cherny *et al.* (2001) *Neuron* 30:655-61), are believed to play a role in dissolving plaques and/or preventing A β aggregation.

3. Reduction of A β accumulation

Based on the strong correlation between A β accumulation, neuronal loss and AD,

- 70 -

- a reduction in A β accumulation should result in decreased plaque formation and minimize neuronal cell death. There are, however, numerous mechanisms and activities which may influence brain A β levels, and these mechanisms can influence many other important cellular functions and processes. For example, production of an intracellular
- 5 C-terminal fragment (CTF) of APP resulting from γ -secretase cleavage between amino acids 49 and 50, close to the cytoplasmic side of the transmembrane domain, is believed to play a role in signal transduction (Pinnix, I *et al.* (2001) *J. Biol. Chem* 276:481-487; Sastre, M. *et al.* (2001) *EMBO Reports* 2(9):835-41; Gu, Y *et al.* (2001) *J. Biol. Chem.* 276(38): 35235-8; Cao, X and Sudhof, T.C. (2001) *Science* 293:115-120). Inhibition of
- 10 such cleavage may result in unwanted side affects. It is, therefore, important when seeking agents for altering A β levels to identify agents that act specifically on the A β endpoint with minimal disruption of other, often overlapping, cellular pathways and processes. Due to the high degree of regulation of and organelle-specific differences in the generation, clearance, and degradation of the various A β peptides, identification of
- 15 agents that target appropriate production enzymes, degradative enzymes, and/or related proteins and receptors involved in A β production and clearance pathways should make possible modulation of the production, clearance and/or degradation of one or more A β peptides without substantially affecting other cellular compositions, processes and activities.
- 20 One approach to treating diseases associated with A β -based amyloidosis, such as Alzheimer's disease, is aimed at reducing A β peptide production by targeting presenilin function. However, because presenilin and presenilin-dependent activities affect substrates other than APP, non-specific modulation (such as, for example, inhibition) of presenilin and/or presenilin-dependent mechanisms can result in unwanted side effects.
- 25 Furthermore, because γ -secretase generates normal non-amyloidogenic peptides, such as p3 and APP CTF, non-specific modulation of γ -secretase may be undesirable. In addition, because release of A β peptides is a normal event in virtually every cell, it may be desirable in some instances to maintain or even elevate levels of particular A β peptides.
- 30 There is a need for agents that modulate the levels of one or more A β peptides of

- 71 -

cells and tissues (intracellular, extracellular, and/or membrane-bound A β), for example, by modulating compositions (*e.g.*, proteases and proteins, such as proteins on which protease activities depend, including presenilins), mechanisms and/or activities involved in A β peptide formation and persistence in cells and/or extracellular medium without

5 substantially affecting (or with only limited or minimal effect on) compositions, mechanisms and/or activities that are not significantly involved in A β peptide formation and persistence. There is particularly a need for agents that modulate the levels of A β 42 peptide in cells and/or extracellular medium without substantially affecting (or with only limited or minimal effect on) compositions, mechanisms, processes and/or activities that

10 are not significantly involved in A β 42 peptide generation and persistence in cells and/or extracellular medium. Such agents have numerous uses. For example, such agents can be used in elucidating the precise elements and pathways involved in A β peptide formation, degradation and clearance in cells. Furthermore, such agents are candidates for the prevention and/or treatment of diseases and disorders involving amyloidosis, such

15 as, for example, AD. Such agents can provide therapeutic and/or prophylactic benefit with limited-to-no potential side effects that can result from non-specific modulation of A β peptide processing and/or clearance.

Provided herein are methods of identifying agents that modulate the levels (including, *e.g.*, cellular and/or extracellular) of one or more A β peptides. In particular

20 embodiments, the methods can be used to identify agents that modulate the levels of A β 42 (including cellular and/or extracellular). In further embodiments, the methods can be used to identify agents that selectively modulate the levels of A β 42 (including cellular and/or extracellular).

In another embodiment, the methods can be used to identify agents that modulate

25 A β peptide levels (and, in particular, A β 42 levels) without substantially affecting (or with limited, minimal or inconsequential effect on) compositions, mechanisms, processes and/or activities that are not significantly involved in the generation, degradation and/or clearance of one or more A β peptides. A composition, mechanism, process or activity that is not significantly involved in the generation, degradation and/or clearance of an A β

30 peptide can be, for example, one that has minimal effect on the generation, degradation

- 72 -

and/or clearance of an A β peptide. Thus, for example, if the generation, degradation and/or clearance of an A β peptide does not differ substantially in the presence and absence of a particular composition, mechanism, process or activity, then the composition, mechanism, process or activity may not be significantly involved in the generation, degradation and/or clearance of an A β peptide. In a particular embodiment, the method involves a step of identifying an agent that modulates the levels (including *e.g.*, cellular and/or extracellular) of one or more A β peptides without substantially altering the substrate-processing activity of presenilin. The method can involve a step of identifying an agent that modulates the levels of one or more A β peptides without substantially altering the cleavage of a presenilin substrate, or portion(s) thereof, that is other than APP. In a further embodiment, the presenilin substrate is LRP. In another embodiment, the method involves a step of identifying an agent that modulates the levels (including, *e.g.*, cellular and/or extracellular) of one or two A β peptides, without substantially altering the levels of one or more other A β peptides. In a particular embodiment, an agent that modulates the levels of A β 42 only, or A β 39 only, or A β 42 and A β 39 only, without substantially altering the levels of one or more other A β peptides, is identified. The agent can be, for example, one that modulates the levels of A β 42 and/or A β 39 without substantially altering the levels of A β 40.

Also provided herein are methods of modulating A β peptide levels (including, *e.g.*, cellular and/or extracellular A β). In one embodiment, the method includes a step of contacting a sample, for example, a cell, with an agent that modulates the level of one or more A β peptides, in particular, A β 42 and/or A β 39, without substantially affecting or altering the level of one or more different A β peptides. For example, the method can include a step of contacting a sample, for example, a cell, with an agent that modulates A β 42 and/or A β 39 levels without substantially altering the levels of A β 40. In another embodiment, the methods include a step of contacting a sample (*e.g.*, a cell) with an agent that modulates the level of one or more A β peptides, particularly A β 42, without substantially affecting a non-APP substrate-processing activity of presenilin. The methods can include a step of contacting a sample with an agent that modulates the level of one or more A β peptides without substantially affecting the cleavage and/or

- 73 -

processing of a presenilin substrate other than APP. In a particular embodiment, the presenilin substrate is LRP.

Further provided herein is an antibody that selectively recognizes A β 42 without substantially binding to other A β peptides. The antibody has numerous uses and provides specific advantages as compared to other antibodies. For example, the antibody can be used in methods of identifying agents that modulate A β 42 levels without substantially affecting the level of other A β peptides. The antibody can further be used in methods of detecting A β 42 in a sample for any purpose, including but not limited to methods of diagnosis of diseases and disorders involving amyloidosis, for example, AD.

Also provided herein are compositions and methods for assessing presenilin activity and/or presenilin-dependent activity. In one embodiment, the methods involved determining the presence or absence and/or level of one or more fragments of LRP and/or the composition of LRP in a sample for which presenilin activity is being assessed. The methods can be used in methods for identifying or screening for agents that modulate presenilin and/or presenilin-dependent activity that are also provided herein. As described herein, presenilins are proteins that are involved in the processing of a number of proteins with various functions and activities, including not only APP but LRP. Because presenilins are involved in diverse reactions with a variety of substrates, it is desirable to identify agents that affect presenilin activity and presenilin-dependent mechanisms. A method provided herein for identifying agents that modulate presenilin and/or presenilin-dependent mechanisms is based on the finding described herein that LRP is a substrate that is processed in a presenilin-dependent mechanism. In one embodiment, the method includes a step of comparing the levels and/or composition of LRP C-terminal fragments in samples containing presenilin that have been contacted with a test agent and samples containing presenilin that have not been contacted with test agent. The methods for identifying an agent that modulates presenilin activity can be applied to methods for identifying candidate agents for the treatment or prophylaxis of a disease or disorder associated with altered presenilin. One embodiment of these methods includes steps of contacting a sample containing LRP and an altered presenilin that is associated with altered LRP processing with a test agent and identifying a candidate

- 74 -

agent that restores LRP processing to that which occurs in the presence of a presenilin that is not associated with altered processing of LRP.

C. Methods of Assessing Presenilin and/or Presenilin-Dependent Activity

Presenilins are transmembrane proteins localized predominantly in the ER and Golgi. Included among the presenilin proteins are the homologous presenilin-1 (PS1) and presenilin-2 (PS2) proteins (see SEQ ID NO: 6 for an amino acid sequence of a PS1 protein and SEQ ID NO: 8 for an amino acid sequence of a PS2 protein). Although the presenilin proteins alone may not have an enzymatic activity, they appear to play an essential role in the proteolytic processing of a variety of proteins, including APP (particularly the γ -secretase cleavage of APP) and in the trafficking and maturation of various cellular proteins (referred to herein collectively as substrates for presenilin activity and/or presenilin-dependent enzyme activity), including, but not limited to Notch, TrkB, APLP2, hIre1 α , E-cadherin and Erb-B4. With respect to processing of APP, it appears that presenilin participates intimately as part of a catalytic complex by which γ -secretase mediates an intramembraneous proteolysis of APP. Two transmembrane aspartate residues (D257 and D385 in PS1; D263 and D366 in PS2) are individually critical for presenilin-associated γ -secretase activity as well as presenilin endoproteolysis.

Inherited mutations in the genes encoding presenilins-1 and -2 account for up to 40% of the early onset cases of familial Alzheimer's Disease (FAD). FAD-associated mutations in PS1 and PS2 give rise to an increased accumulation of A β 42 in AD patients and transfected cell lines and transgenic animals expressing FAD mutant forms of PS1 or PS2.

Because presenilins and presenilin-dependent activities play a key, yet mechanistically unresolved, role in the cleavage of numerous proteins involved in a variety of processes (some of which are associated with diseases such as Alzheimer's Disease), there is a need for compositions and methods that can be used in assessing presenilin activity. For example, assessment of presenilin activity using such compositions and methods can greatly facilitate the elucidation of the mechanisms of protein processing in normal and disease states and the determination of the number,

- 75 -

specificities, regulation and potential overlap of the proteolytic activities that function in the cleavage of an array of transmembrane proteins. Furthermore, compositions and methods for the assessment of presenilin activity may also be used in screening of agents that specifically modulate various presenilin-dependent enzyme activities. Such agents
5 may also be of use in elucidating the mechanisms of protein processing in normal and disease states. In addition, such agents can be candidate agents for the prevention and/or treatment of diseases associated with altered proteolytic processing of cellular proteins, such as, for example, diseases involving amyloidosis, including AD.

Provided herein are compositions and methods for assessing presenilin activity
10 and/or presenilin-dependent activity. In one embodiment, the methods involve determining the level of one or more fragments of LRP and/or the composition of LRP in the presence of a sample for which presenilin activity is being assessed. Determining the level can be determining the presence or absence of one or more fragments as well as making a more quantitative assessment of amount of the fragment. The methods are
15 based on the finding described and demonstrated herein that the low density lipoprotein receptor-related protein (LRP) is processed by a presenilin-dependent enzyme activity. In a particular method for assessing presenilin activity, the level of a fragment of LRP that is from a C-terminal portion of LRP is determined, such as, for example, an approximately 20 kD-fragment. Also provided is a method of identifying agents that
20 modulate presenilin activity and/or presenilin-dependent activity which involve comparing, in the presence and absence of test agents, the level of one or more fragments of LRP and/or composition of LRP in the presence of a presenilin activity. In a particular embodiment, the level of a fragment of LRP that is from a C-terminal portion of LRP is determined, such as, for example, an approximately 20 kD-fragment. Determining the
25 level for any of these methods can be determining the presence or absence of one or more fragments as well as making a more quantitative assessment of amount of the fragment.

Further provided is a method for identifying candidate agents for the treatment and/or prevention of a disease or disorder, such as a disease or disorder associated with altered presenilin function or activity, which includes a step of comparing, in the
30 presence and absence of test agents, the level of one or more fragments of LRP and/or

- 76 -

composition of LRP in the presence of a presenilin encoded by a mutant or polymorphic nucleic acid. In a particular embodiment, the level of a fragment of LRP that is from a C-terminal portion of LRP is determined, such as, for example, an approximately 20 kD-fragment. In particular embodiments, the disease or disorder is associated with amyloidosis, for example, Alzheimer's disease. The mutant nucleic acid can be, for example, one that encodes a presenilin that is linked to Alzheimer's disease. For example, the mutant nucleic acid may encode any one or more of the at least 60 mutations in human PS1 and the at least two mutations in human PS2 that have been genetically linked to early onset familial Alzheimer's disease (FAD) (see, e.g., Van Gassen *et al.* (2000) *Neurobiol. Dis.* 7:135-151; Checler (1999) *IUBMB Life* 48:33-39; St. George-Hyslop (2000) *Biol. Psychiatry* 47:183-199; Steiner *et al.* (1999) *Eur. Arch. Psychiatry Clin. Neurosci.* 249:266-270). Included among such mutations are the PS2 FAD mutation N141I (Volga German FAD mutant) and the PS1 FAD mutation M146L.

Additional methods of assessing presenilin activity and/or presenilin-dependent enzyme activity involve determining the levels and/or compositions of fragments of other presenilin substrates. Presenilin substrates are peptides, polypeptides, proteins or fragments thereof that are proteolytically processed, at least in part, in a presenilin-dependent manner. Thus, if presenilin is absent, or presenilin activity is inhibited or reduced, the proteolytic processing of a presenilin substrate is altered, for example by an alteration in the levels and/or composition of fragments generated from the substrate, relative to the proteolytic processing of the substrate that occurs in the presence of normal (e.g., wild-type) presenilin activity. Generally, a presenilin substrate can contain about one transmembrane domain, an ectodomain that is released or shed into the extracellular medium, and/or an intracellular domain. Typically, processing of a presenilin substrate includes an initial cleavage of the substrate (typically by a metalloprotease) at a site located in the extracellular domain of the substrate to release an ectodomain of the substrate, followed by presenilin-mediated cleavage of the remaining membrane-bound portion of the substrate to yield an intracellular fragment, which may be translocated to the nucleus of a cell.

30 1. LRP Assay

- 77 -

a. LRP

Low density lipoprotein receptor-related protein (LRP) is a cell surface receptor that binds and internalizes a number of diverse extracellular ligands, including apolipoprotein E (apoE), α 2-macroglobulin (α 2M), APP, tissue-type plasminogen activator (tPA) and lactoferrin, for degradation by lysosomes. LRP expression is widespread; however, it is most highly expressed in the liver, brain and placenta. With respect to its expression in the brain, LRP is primarily a neuronal receptor expressed in the cortex and hippocampus and is also expressed in activated astrocytes, glia and microglia. Mature LRP is a heterodimer containing an N-terminal 515 kD extracellular subunit (α chain) and a C-terminal 85 kD membrane-anchored subunit (β chain) which are non-covalently associated. The mature receptor is generated by proteolytic cleavage of a 600 kD precursor polypeptide in a trans-Golgi compartment in a process that involves the endoprotease furin. The amino acid sequence of the LRP precursor polypeptide is provided in SEQ ID NO: 10 (see also GenBank Accession No. Q07954), and DNA encoding the polypeptide is provided in SEQ ID NO: 9. Proteolytic processing of precursor LRP to yield the mature receptor occurs at amino acid position 3925 C-terminal to the tetrabasic amino acid sequence RHRR. LRP is anchored in the plasma membrane by a single transmembrane domain, and its cytoplasmic tail includes two copies of the internalization signal NPXY.

Additionally, LRP undergoes another proteolytic processing step at the cell surface which involves a metalloproteinase (Quinn *et al.* (1999) *Exp. Cell. Res.* 251:433-441). This processing results in "shedding" from the cell surface of a portion of LRP containing the α chain (an ~500-kD soluble polypeptide) that is noncovalently associated with a truncated β chain (the extracellular portion of the β chain (i.e., amino acids 3944-4420 of SEQ ID NO: 10; Mr ~67 kD or Mr ~55 kD after deglycosylation with *N*-glycosidase F).

LRP is a member of the low-density lipoprotein receptor (LDLR) family. The extracellular region of receptors in this family contains several structural modules which include ligand-binding repeats of ~40 amino acids (including six cysteine residues forming three disulfide bonds), epidermal growth factor (EGF) precursor repeats (each

- 78 -

also containing six cysteine residues), and modules with a consensus tetrapeptide (YWTD). In addition to these modules, these receptors contain a single transmembrane domain and a relatively short cytoplasmic tail with endocytosis signals and elements for interaction with cytoplasmic adaptor and scaffold proteins (*e.g.*, Dab, FE65, c-jun N-terminal kinase interacting proteins (JIPs) and postsynaptic density protein PSD-95) for mediating signal transduction.

LRP may have a significant role in the pathogenesis of AD. Several LRP ligands, including apoE, lactoferrin and α 2M, bind A β . Such ligand interactions are believed to facilitate A β clearance through an LRP-mediated endocytic pathway (Qiu *et al.* (1999) *J. Neurochem.* 73:1393-8). LRP levels are reduced in AD and in transgenic mice expressing presenilin and cells transfected with presenilin-encoding DNA. Furthermore, transgenic mice overexpressing the M146L or L286V presenilin-1 mutations associated with AD reportedly have decreased levels of LRP expression in certain neuronal populations. LRP also interacts with APP via adaptor proteins, such as FE65. In addition, genetic association studies indicate that the LRP gene may be a susceptibility locus for late-onset AD.

b. LRP is processed by a presenilin-dependent activity

As described and demonstrated herein (see the EXAMPLES), LRP is processed by a presenilin-dependent enzyme activity. LRP processing was analyzed in cell lines expressing defective (*i.e.*, loss of function) PS1 proteins encoded by nucleic acid lacking exons 1 and 2 (see, *e.g.*, GenBank Accession No. L76518 for sequences of exons 1 and 2) of the PS1-encoding DNA or nucleic acid coding for an alanine instead of an aspartic acid residue at amino acid 385 (D385A), which is essential to PS1 function. These cells had been generated by transfecting mouse neuroblastoma (N2a) cells (see, *e.g.*, ATCC, Rockville, MD), which express endogenous LRP, with nucleic acid encoding wild-type human APP695 and nucleic acid encoding human PS-1 (wild-type, D385A mutant, or exon 1 and 2 deletion). It was discovered that LRP processing is altered in the cells expressing defective PS1 proteins relative to cell lines expressing normal wild-type PS1. Specifically, an ~20-kD peptide was detected in an immunoassay of lysates of cells that had been transfected with mutant PS1-encoding DNA that was not detected (or detected

- 79 -

at much lower levels) in lysates of cells that had been transfected with wild-type PS1-encoding DNA. The detection antibody (R9377) was one generated against the carboxyl-terminal 13 amino acids of human LRP. Because the ~20-kD peptide from a C-terminal portion of LRP, which contains an epitope recognized by an antibody generated against the C-terminal 13-amino acids of LRP, was absent or only barely detectable in lysates of cells expressing a wild-type PS1, but present at readily detectable levels in lysates of cells that contain mutant PS1 protein, it appears that a PS1-dependent activity cleaves LRP in such a way as to eliminate an amino acid sequence in a C-terminal region of LRP that is recognized by C-terminus-reactive antibody. The processing of APP and Notch, two substrates for presenilin-dependent processing activity, was also analyzed in these cells, in addition to the analysis of LRP processing. Analogous results, in which particular C-terminal fragments of APP and Notch were detected in lysates of PS1 mutant cells but not in lysates of wild-type PS1 cells, were obtained in analyses of APP and Notch processing. Thus, the results revealed a concordance of the activity of PS-1 with the three substrates. The similar findings support a conclusion of a presenilin-dependent cleavage of LRP. It was also found that the LRP β chain alone is sufficient for processing by PS-1, and that trafficking to the plasma membrane is a necessary event for the normal processing of LRP by the PS-1 active complex.

As also described in the EXAMPLES, in the presence of the γ -secretase inhibitor DAPT (N-[N-(3,5-difluorophenacetyl)-L-alanyl]-S-phenylglycine-*t*-butyl ester), an accumulation of an approximately 20 kD fragment of LRP that is from a C-terminus portion of LRP is observed. The fragment is one that is recognized and bound by a polyclonal antibody (*e.g.*, antibody R9377 as described in the EXAMPLES) generated against a carboxyl-terminal peptide (the carboxyl-terminal 13 amino acids) of human LRP (C-GRGPEDEIGDPLA) with N-terminal cysteine added for conjugation to ovalbumin. The accumulation of the ~20-kD fragment from a C-terminal portion of LRP parallels the accumulation of APP C-terminal fragments (CTFs). This finding indicates that LRP fragment accumulation is a measure of presenilin/ γ -secretase activity.

Advantages of using LRP fragment analysis in a method for assessing presenilin activity include: (1) LRP is highly expressed in adult brain, (2) the analysis is easily amenable to

- 80 -

testing *in vivo* samples, (3) endogenous LRP is expressed at sufficient levels in cell culture models such that transfection the cells with nucleic acid encoding LRP in order to increase expression levels for detection is not necessary, and (4) LRP appears to have a significant role in the pathogenesis of AD. Furthermore, LRP has been shown to have a
5 potentially significant role in the clearance of A β as described above.

c. Methods of modulating LRP

Because of the involvement of LRP in critical cellular processes, including, but not limited to, signal transduction and receptor-mediated endocytosis, and in mechanisms associated with Alzheimer's disease, there is a need for methods and compositions that
10 can be used in modulating LRP. LRP modulation can be any alteration of LRP, including, but not limited to, any alteration in the processing, structure, function (including, for example ligand-binding) and/or activity (including, for example, signal transduction and receptor-mediated endocytosis) of LRP. Modulation of LRP has numerous uses. For example, the ability to modulate LRP can greatly facilitate the
15 elucidation and detailed characterization of the mechanisms involved in signal transduction and receptor-mediated endocytosis. Furthermore, modulation of LRP has applications in the treatment and prophylaxis of diseases of signal transduction and endocytosis, as well as AD.

As described and demonstrated herein, LRP is processed by a presenilin-
20 dependent enzyme activity. The processing of LRP can have significant effects on its structure, function and activity.

Provided herein are methods for modulating LRP. The LRP can be in a sample that has been selected for LRP modulation. Such samples include, but are not limited to, cells, tissues, organisms, lysates, extracts and membrane preparations of cells and cell-
25 free samples containing LRP, including, for example, extracellular medium, tissue and body fluids. In one embodiment, the methods involve altering the structure, function and/or activity of a presenilin (and/or fragments thereof) in a sample containing LRP, and/or fragment(s) thereof, and a presenilin, and/or fragment(s) thereof, whereby the LRP is modulated. The structure, function and/or activity of a presenilin can be altered in a
30 number of ways which can vary depending in large part on the sample. For example, the

- 81 -

function and activity of presenilins (particularly functions and activities relating to interaction of presenilin with other molecules) can be altered by contacting presenilin with antibodies, and/or fragment(s) thereof, that bind presenilin, particularly antibodies that bind to presenilin in such a way as to impede or eliminate the ability of presenilin to interact with binding partners. If the sample is a cell, the function and/or activity of presenilin in the cell can be altered, for example, by enhancing, increasing, reducing or eliminating the expression of the presenilin. Methods are known in the art for transferring nucleic acids encoding presenilin into cells and for reducing or eliminating the expression of functional proteins, such as presenilin, in cells (*e.g.*, gene knock-out, antisense RNA and RNA interference techniques).

In another embodiment, the methods involve contacting a sample containing an LRP, and/or fragment(s) thereof, and presenilin, and/or fragment(s) thereof, with an agent that modulates presenilin or presenilin-dependent activities. The sample is one that has been selected for LRP modulation. An agent that modulates presenilin or presenilin-dependent activities can be identified using methods provided and described herein.

d. Assessment of presenilin activity based on LRP

In a method for assessing presenilin activity provided herein, the level of one or more fragments of LRP and/or the composition of LRP is determined for a sample for which presenilin activity is being assessed. Examples of a sample for which presenilin activity is being assessed include, but are not limited to, a cell that expresses presenilin, a lysate or extract of a cell that expresses presenilin, or membranes prepared from a cell that expresses presenilin. The cell can endogenously express presenilin and/or express heterologous presenilin. LRP can be added to the sample or can be expressed endogenously and/or heterologously by the cell. In a particular embodiment, the method includes assessing presenilin activity of a cell by evaluating the level (which includes determining the presence or absence of) of a fragment from a C-terminal portion of LRP in a cell lysate.

To assess presenilin activity in these methods, the processing of LRP is evaluated. In evaluating LRP processing, the composition of LRP can be evaluated. The composition of LRP refers to the make-up of any LRP that is present anywhere in the

- 82 -

analysis mixture, including the assay medium in which the analysis is being performed, an extracellular medium, or a cell membrane, lysate or extract. Thus, in one embodiment, the structure of any LRP present can be evaluated to, for example, determine whether LRP is intact or has been processed and appears as a fragment or
5 fragments of sizes smaller than the intact LRP molecule or than either one or both of the intact chains of LRP. In evaluating LRP processing, the levels (including the presence or absence) of one or more LRP fragments can be determined.

In particular, the LRP composition is evaluated to determine if any fragment(s) indicative of presenilin-dependent cleavage or altered presenilin-dependent cleavage of
10 LRP are present and/or the level of any such fragment(s). A presenilin-dependent cleavage described herein occurs within the C-terminal portion of LRP and within the β chain. Thus, a presenilin-dependent cleavage of LRP can be one that occurs in the C-terminal portion of LRP at a position C-terminal to amino acid position 3925 of SEQ ID NO: 10 (or of the amino acid sequence provided as GenBank Accession No. Q07954).
15 The presenilin-dependent cleavage of LRP can be one that occurs within the sequence of the last approximately 580, 500, 450, 400, 350, 300, 250, 200, 150, 100, 50, 25, or less amino acids of LRP. The presenilin-dependent cleavage can be one that occurs C-terminal to the extracellular portion of the β chain (i.e., approximately amino acids 3944-4420 of SEQ ID NO: 10 or of the amino acid sequence provided as GenBank Accession
20 No. Q07954); thus, C-terminal to about amino acid 4420 of SEQ ID NO: 10. The presenilin-dependent cleavage of LRP can be one that occurs near or within the region of the LRP protein that extends from a point located intracellularly and adjacent to the cytoplasmic face of the cell membrane to a point located within the cell membrane. Such a presenilin-dependent cleavage of LRP can be one that generates a soluble intracellular
25 peptide, containing the extreme C-terminus of LRP, and a membrane-associated peptide containing amino acid sequence of the transmembrane region of LRP, particularly the more C-terminal region of the transmembrane segment of LRP. Any LRP fragments generated by such presenilin-dependent activities have a molecular weight that is less than that of the β chain of LRP (β chain molecular weight is approximately 85-90 kD, or
30 approximately 67 kD after deglycosylation with *N*-glycosidase F). In particular

- 83 -

embodiments, an LRP fragment generated by such presenilin-dependent activities has a molecular weight that is less than that of the extracellular portion of the β chain of LRP (the extracellular portion of the β chain molecular weight is approximately 67 kD, or approximately 55 kD after deglycosylation with *N*-glycosidase F). Thus, an LRP

5 fragment generated by a presenilin-dependent cleavage can have a molecular weight that is, for example, less than about 85 kD, 80 kD, 75 kD, 70 kD, 65 kD, 60 kD, 55 kD, 50 kD, 45 kD, 40 kD, 35 kD, 30 kD, 25 kD, 20 kD, 15 kD or 10 kD or less. LRP fragments that are particularly indicative of a presenilin-dependent cleavage have a molecular weight that is less than about 15 kD, 13 kD, 12 kD, 10 kD or 5 kD.

10 In a particular embodiment of a method for assessing presenilin activity provided herein, LRP processing in a sample for which presenilin activity is being assessed can be evaluated by evaluating the LRP composition to determine if any fragment(s) indicative of altered presenilin-dependent cleavage of LRP are present and/or the level of any such fragment(s). Altered presenilin activity can be, for example, an increase, reduction or
15 elimination of presenilin activity. In a particular embodiment of this method, the presence or absence and/or the level of an LRP fragment that is cleaved in the presence of a presenilin-dependent activity (*e.g.*, presenilin-dependent γ -secretase activity), and thus absent (or present at low levels) in the presence of the presenilin-dependent activity, but that can be detected intact when the presenilin-dependent activity is altered (such that
20 it is reduced or eliminated) is assessed. One such fragment indicative of altered presenilin-dependent cleavage has a molecular weight of between about 25 kD and 15 kD, and, in particular, about 20 kD. The fragment can be one that is cleaved in the presence of a presenilin-dependent activity in such a way as to eliminate an amino acid sequence in a C-terminal region of LRP that is recognized by C-terminus-reactive
25 antibody (*i.e.*, the cleavage in the presence of a presenilin-dependent activity eliminates an epitope in the fragment that is recognized by an antibody generated against the C-terminal 13-amino acids of LRP). The LRP fragment can be one that contains an amino acid sequence located within the sequence of amino acids 4420-4544 of SEQ ID NO:10.

As described herein above, LRP is also cleaved by activities that are not
30 presenilin dependent. Specifically, in one cleavage that is not a presenilin-dependent/ γ -

- 84 -

secretase activity, mature LRP, i.e., separate, but noncovalently associated, α (N-terminal 515 kD extracellular subunit) and β (C-terminal 85 kD membrane-anchored subunit) chains, is generated by proteolytic cleavage at amino acid position 3925 (C-terminal to the tetrabasic amino acid sequence RHRR) of the 600-kD precursor polypeptide (see
5 SEQ ID NO: 10 and GenBank Accession No. Q07954) in a process that involves the endoprotease furin. Another cleavage of LRP that is not presenilin-dependent is the metalloproteinase-mediated proteolytic processing at the cell surface which results in "shedding" from the cell surface of a portion of LRP containing the α chain (an ~500-kD soluble polypeptide) that is noncovalently associated with a truncated β chain (the
10 extracellular portion of the β chain (i.e., amino acids 3944-4420 of SEQ ID NO: 10; Mr ~67 kD or Mr ~55 kD after deglycosylation with *N*-glycosidase F). A fragment such as these that does not result from a presenilin-dependent cleavage generally is not alone indicative of presenilin activity.

In methods for assessing presenilin activity provided herein that include a step of
15 determining the level of one or more fragments of LRP and/or LRP composition, LRP protein and/or fragments thereof can be detected and/or measured by any method known to those of skill in the art for measuring protein level or by any method described herein. In a particular embodiment of the method, LRP protein or a peptide fragment thereof is detected by immunoassay. For example, an LRP fragment from a C-terminal portion of
20 LRP is visualized by immunoblotting of cell lysates with the anti-LRP polyclonal antibody (R9377) prepared to the carboxyl-terminal 13 amino acid peptide of LRP (C-GRGPEDEIGDPLA) as described in the EXAMPLES.

e. Methods for identifying or screening for agents that modulate presenilin activity

25 Methods for assessing presenilin activity provided herein can be applied to the identification of or screening for agents that modulate presenilin activity. One method provided herein for identifying or screening for agents that modulate presenilin activity includes steps of contacting a sample containing a presenilin and a lipoprotein receptor-related protein (LRP) and/or portion(s) or fragment(s) thereof with a test agent and
30 identifying an agent that alters the processing and/or cleavage of an LRP or fragment

- 85 -

thereof.

A sample that can be used in the methods of identifying an agent that modulates presenilin activity can be any composition (*e.g.*, a biological or physiological composition) that includes a source of presenilin and a source of LRP and/or portion(s) thereof. Examples of samples include, but are not limited to, a cell, a cell extract or lysate, a cellular membrane and a cell-free medium.

(1) Presenilin and LRP (and/or portion(s) thereof)

Sources of presenilin and LRP include, but are not limited to: a cell that expresses endogenous or heterologous presenilin and/or LRP; a cell that expresses a recombinant portion(s) or fragment(s) of presenilin and/or LRP; lysates, extracts, or membrane fractions of any such cells; presenilin, LRP, or a portion thereof, that is isolated from such cells; and synthetic presenilin or LRP protein or synthetic proteins that represent a portion of presenilin or LRP.

Compositions, and methods of making compositions, that are sources of presenilin, LRP, and portion(s) thereof, are described herein and known in the art. For example, cells that endogenously express presenilin and/or LRP are known in the art as are nucleic acids encoding presenilin (see, *e.g.*, SEQ ID NOs: 5 and 7) and LRP (see, *e.g.*, SEQ ID NO: 9) that can be used to express the encoded proteins in cells. Methods of preparing lysates, extracts and membrane fractions of such cells are also described herein and known in the art, as are synthetic methods for generating proteins and peptides and preparatory methods of isolating proteins and peptides.

(2) Identifying an agent that alters the processing and/or cleavage of LRP (or portion(s) thereof)

In general, the step of identifying an agent that alters the processing and/or cleavage of LRP (or portion(s) thereof) can involve a comparison of the cleavage and/or processing of LRP (and/or portion(s) thereof) in a sample that has been contacted with the test agent (*i.e.*, test sample) and in a sample that has not been contacted with the test agent (*i.e.*, control sample). If the cleavage and/or processing of LRP (and/or portion(s) thereof) in the test and control samples differs, then the agent is identified as one that modulates presenilin activity. For example, processing of LRP and/or the level of a

- 86 -

particular fragment of LRP in the test and control samples may differ by at least 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75% or more than 75%. The control sample can be the same physical sample as the test sample or a different sample. When the control and test samples are the same, the control is the sample in the absence of test agent.

The processing or cleavage of an LRP or fragment(s) thereof can be assessed by determining the presence or absence and/or level of one or more fragments of LRP and/or the composition of LRP using, for example, materials and methods described herein. Thus, the LRP composition can be evaluated to determine if any fragment(s) indicative of presenilin-dependent cleavage of LRP or altered presenilin-dependent cleavage of LRP are present and/or the level of any such fragments. Such fragments and compositions are described herein. In a particular embodiment, the processing or cleavage of an LRP or fragment(s) thereof is assessed by determining the presence or absence and/or level of an LRP fragment that is cleaved in the presence of a presenilin-dependent activity (presenilin-dependent γ -secretase activity), and thus absent (or present at low levels) in the presence of the presenilin-dependent activity, but that can be detected intact when the presenilin-dependent activity is altered (such that it is eliminated or reduced). In one embodiment, the presence or absence and/or level of an LRP fragment having a molecular weight of between about 25 kD and 15 kD, and, in particular, about 20 kD, is assessed. Typically, the ~ 20 kD fragment is one that is present when an LRP is not cleaved by a presenilin-dependent activity, such as one that occurs in the presence of an inhibitor of a presenilin-dependent activity such as DAPT. In a particular embodiment, the fragment is from a C-terminal portion of LRP, i.e., a CTF. The LRP fragment can be one that contains an amino acid sequence located within the sequence of amino acids 4420-4544 of SEQ ID NO:10. In a further embodiment, the fragment is one that is recognized by an antibody generated against C-terminal amino acids (e.g., the C-terminal 13 amino acids) of LRP, such as, for example, the polyclonal antibody R9377 described herein.

The methods for identifying an agent that modulates presenilin activity as provided and described herein can be applied to the identification of candidate agents for

- 87 -

the treatment or prophylaxis of a disease associated with an altered presenilin. A particular embodiment of this method includes steps of contacting a sample containing a lipoprotein receptor-related protein (LRP), and/or fragment(s) thereof, and an altered presenilin, and/or fragment(s) thereof, that is associated with an altered processing of LRP with a test agent and identifying a candidate agent that restores LRP processing substantially to the processing that occurs in the presence of a presenilin, and/or fragment(s) thereof, that is not associated with an altered processing of LRP. The altered presenilin, and/or fragment(s) thereof, can be one that has an altered function or activity. Altered presenilins include, for example, a presenilin and/or fragment(s) thereof containing a mutation and/or encoded by a polymorphic nucleic acid that contains a mutation. Thus, the altered presenilin and/or fragment(s) thereof, can be one that is altered relative to a wild-type presenilin. Typically, a wild-type protein, such as, for example, a presenilin protein, can be one that is encoded by a predominant allele in a population or any allele that is not associated with disease or a pathogenic condition. A wild-type presenilin can be one that occurs in an organism that exhibits normal presenilin-dependent LRP processing patterns. The altered presenilin can be, for example, one that is encoded by a nucleic acid linked to Alzheimer's disease. For example, the nucleic acid may include any one or more of the at least 60 mutations in human PS1 and the at least two mutations in human PS2 that have been genetically linked to early onset familial Alzheimer's disease (FAD). Exemplary presenilins with altered activity include FAD-associated mutant forms of PS1 and PS2 that give rise to an increased accumulation of A β 42 in AD patients and transfected cell lines and transgenic animals in which they are expressed. Included among such mutations are the PS2 FAD mutation N141I (Volga German FAD mutant) and the PS1 FAD mutation M146L.

Examples of diseases associated with an altered presenilin for which the methods provided herein can be used to identify candidate therapeutic or prophylactic agents include, but are not limited to, amyloidosis-associated diseases and neurodegenerative diseases. In a particular aspect, the disease is Alzheimer's Disease.

The sample used in the methods can be any sample, including samples described herein for the methods of identifying agents that modulate presenilin activity. For

- 88 -

example, a sample can contain cell(s), tissue, a cell or tissue lysate or extract, a body fluid, a cell membrane or composition containing cell membranes and a cell-free extract or other cell-free sample. In a particular embodiment, the sample includes a cell that contains the presenilin and LRP.

5 In general, the step of identifying a candidate agent that restores LRP processing to the processing that occurs in the presence of a presenilin that is not associated with an altered processing of LRP can involve a comparison of the cleavage and/or processing of LRP (and/or portion(s) thereof) in a sample that has been contacted with the test agent (i.e., test sample) and in a sample that has not been contacted with the test agent (i.e.,
10 control sample). If the cleavage and/or processing of LRP (and/or portion(s) thereof) in the test and control samples differs, then the test agent is identified as a candidate agent for the treatment and/or prophylaxis of a disease associated with an altered presenilin. For example, processing of LRP and/or the level of a particular fragment of LRP in the test and control samples may differ by at least 10%, 15%, 20%, 25%, 30%, 35%, 40%,
15 45%, 50%, 55%, 60%, 65%, 70%, 75% or more than 75%. Additionally or alternatively, the cleavage and/or processing of LRP in the test sample can be compared to that in a positive control sample. An example of a positive control is a sample containing LRP (and/or portion(s) thereof) and a presenilin that is not associated with an altered processing of LRP (or an unaltered or wild-type presenilin). In comparing the test
20 sample to the positive control, a test agent is identified as a candidate agent for the treatment and/or prophylaxis of a disease if the cleavage and/or processing of LRP (and/or portion(s) thereof) in the test and positive control samples is substantially similar. LRP cleavage and/or processing in the test and positive control samples could be
25 substantially similar if the LRP processing and/or cleavage in the test sample is more similar to that in the positive control sample than that in the control sample that contains the altered presenilin and that was not contacted with the test agent.

 The cleavage and/or processing of LRP (and/or portion(s) thereof) in a sample can be assessed, for example, using any of the methods and compositions provided and described herein. Assessing cleavage and/or processing of LRP can provide an
30 assessment of presenilin activity. The processing and/or cleavage of an LRP can be

- 89 -

assessed by determining the presence or absence and/or level of one or more fragments of LRP and/or the composition of LRP. In a particular embodiment, the processing or cleavage of the LRP or fragment(s) thereof is assessed by determining the presence or absence and/or level of an LRP fragment that has a molecular weight of between about 5 25 kD and 15 kD, and, in particular, about 20 kD. The fragment can be one that is contained within a transmembrane region of LRP and/or binds with an antibody generated against a C-terminal amino acid sequence of an LRP, such as, for example, a sequence of about the C-terminal 13 amino acids of an LRP. The LRP fragment can be one that contains an amino acid sequence located within the sequence of amino acids 10 4420-4544 of SEQ ID NO:10. The fragment can be one that is present when an LRP is not cleaved by a presenilin-dependent activity, for example, as may occur in the presence of an inhibitor of a presenilin-dependent activity such as, for example, DAPT. In a further embodiment, the fragment is one that is recognized by an antibody generated against C-terminal amino acids (*e.g.*, the C-terminal 13 amino acids) of LRP, such as, for 15 example, the polyclonal antibody R9377 described herein.

2. Notch NICD Assay

Notch is a single transmembrane domain cell surface receptor that facilitates many cell fate decisions during development, including neurogenesis. Although its function in mature cells is unclear, its presence in adult mammalian brain has been 20 demonstrated, although at significantly lower levels than in embryonic brain (Berezovska *et al.*, 1998, *J. Neuropathol Exp Neurol.* 57(8):738-45). In addition, a potential role in adult brain including neurite extension has been suggested (Berezovska *et al.*, 1999, *Brain Res. Mol. Brain Res.* 69(2):273-80). Notch, as well as APP, has been found to form stable complexes with PS1 in transfected mammalian cells (Xia, W. *et al.*, 1997, 25 *Proc. Natl. Acad. Sci.* 94:8208-8213; Ray, W.J., *et al.*, 1999, *Proc. Natl., Acad. Sci.* 96:3263-3268).

Notch is synthesized as a 300 kDa precursor molecule, full-length notch (FLN), and undergoes at least three different proteolytic processing events during maturation and signal transduction. The amino acid sequence of the notch precursor polypeptide is 30 provided in SEQ ID NO: 32, and DNA encoding the polypeptide is provided in SEQ ID

- 90 -

NO: 31. In the trans-Golgi network lumen, FLN is cleaved by the protease Furin at a site in the extracellular domain. This cleavage generates two fragments that remain associated during transport to the cell surface forming a heterodimeric receptor at the cell surface. Ligand binding to the receptor triggers an additional cleavage of the
5 extracellular region of the C-terminal domain shortening the extracellular region to 12 amino acids. A third presenilin-dependent proteolytic cleavage event occurs within the transmembrane domain and releases the nuclear intracellular carboxyl domain (NICD). A presenilin-dependent cleavage of Notch has been shown between residues G1743 and V1744 (SEQ ID NO: 32 or the amino acid sequence provided as GenBank Accession No.
10 AF308602). NICD translocates to the nucleus and activates transcription of target genes that influence crucial cell fate decisions during development and particularly haematopoiesis.

In a method for assessing presenilin activity provided herein, the level of one or more fragments of Notch and/or the composition of Notch is determined for a sample for
15 which presenilin activity is being assessed. Examples of a sample for which presenilin activity is being assessed include, but are not limited to, a cell that expresses presenilin, a lysate or extract of a cell that expresses presenilin, or membranes prepared from a cell that expresses presenilin. The cell can endogenously express presenilin and/or express heterologous presenilin. Notch can be added to the sample or can be expressed
20 endogenously and/or heterologously by the cell. In a particular embodiment, the method includes assessing presenilin activity of a cell by evaluating the level (which includes determining the presence or absence of) of a fragment from a C-terminal portion of Notch in a cell lysate.

To assess presenilin activity in these methods, the processing of Notch is
25 evaluated. In evaluating Notch processing, the composition of Notch can be evaluated. The composition of Notch refers to the make-up of any Notch that is present anywhere in the analysis mixture, including the assay medium in which the analysis is being performed, an extracellular medium, or a cell membrane, lysate or extract. Thus, in one embodiment, the structure of any Notch present can be evaluated to, for example,
30 determine whether Notch is intact or has been processed and appears as a fragment or

- 91 -

fragments of sizes smaller than the intact Notch molecule. In evaluating Notch processing, the levels, and/or the presence or absence, of one or more Notch fragments can be determined.

In particular, the Notch composition is evaluated to determine if any fragment(s) indicative of presenilin-dependent cleavage (or altered presenilin-dependent cleavage) of Notch are present and/or the level of any such fragment(s). A presenilin-dependent cleavage described herein occurs within the C-terminal portion of Notch. Thus, a presenilin-dependent cleavage of Notch can be one that occurs in the C-terminal portion of Notch at a position C-terminal to amino acid position 1743 of SEQ ID NO: 32 (or of the amino acid sequence provided as GenBank Accession No. AF308602). The presenilin-dependent cleavage of Notch can be one that occurs within the sequence of the last approximately 850, 815, 800, 750, 700, 750, 700, 650, 600, 650, 600, 550, 500, 450, 400, 350, 300, 250, 200, 150, 100, 50, 25, or less amino acids of Notch. The presenilin-dependent cleavage can be one that occurs C-terminal to the extracellular portion of Notch (i.e., C-terminal to amino acid 1727 of SEQ ID NO: 32 or of the amino acid sequence provided as GenBank Accession No. AF308602). The presenilin-dependent cleavage of Notch can be one that occurs near or within the region of the Notch protein that extends from a point located intracellularly and adjacent to the cytoplasmic face of the cell membrane to a point located within the cell membrane. Such a presenilin-dependent cleavage of Notch can be one that generates a soluble intracellular peptide containing the extreme C-terminus of Notch and a membrane-associated peptide containing amino acid sequence of the transmembrane region of Notch, particularly the more C-terminal region of the transmembrane segment of Notch. A Notch fragment generated by a presenilin-dependent cleavage can be one that can be detected by a reagent that binds to or recognizes an amino acid sequence from a C-terminal portion of Notch.

As described herein above, Notch is also cleaved by activities that are not presenilin dependent. Specifically, in one cleavage that is not a presenilin-dependent/ γ -secretase activity, Notch is cleaved at the cell surface which results in "shedding" from the cell surface of a portion of the extracellular segment of Notch. A fragment such as

- 92 -

this that does not result from a presenilin-dependent cleavage generally is not alone indicative of presenilin activity.

In particular embodiments, Notch processing by PS1/ γ -secretase can be assessed by determining the levels and/or presence or absence of the Notch ICD peptide and/or the Notch membrane-associated peptide that result from presenilin-dependent cleavage of Notch. In addition, the level, presence or absence of a Notch fragment that occurs in the absence of presenilin-dependent cleavage of Notch can be determined. Notch peptide levels can be measured by any method known to those of skill in the art for measuring protein level or by any method described herein. In a particular embodiment of the method, Notch peptide levels are measured by immunoassay. Anti-Notch peptide antibodies for use in such immunoassays can be obtained by the methods described herein or known to those of skill in the art. For example, Myc-tagged Notch derivatives may be used and detected with monoclonal anti-Myc antibodies (i.e., 9E10 from ATCC) (Schroeter *et al.*, (1998) *Nature* 39: 382-386; Song *et al.*, (1999) *Proc. Natl. Acad. Sci.* 96: 6959-6963) or V5 antibody epitope tagged Notch derivatives may be used and detected with anti-V5 antibody as described in the EXAMPLES.

3. E-cadherin assay

E-cadherin controls a wide array of cellular behaviors including cell-cell adhesion, differentiation and tissue development. Presenilin has been shown to form complexes with the cadherin/catenin adhesion system resulting in cleavage and release of the E-cadherin intracellular domain and disassembly of adherens junctions (Baki *et al.* 2001, *Proc. Natl. Acad. Sci.* 98(5):2381-2386; Marambaud *et al.* 2002, *EMBO J.* 21(8):1948-56). The amino acid sequence encoding a full-length human E-cadherin polypeptide is provided in SEQ ID NO: 34, and DNA encoding the polypeptide is provided in SEQ ID NO: 33. A presenilin-1-dependent γ -secretase cleavage stimulated by apoptosis or calcium influx occurs between human E-cadherin residues Leu731 and Arg732 at the membrane-cytoplasm interface. The PS1/ γ -secretase system cleaves both the full-length E-cadherin and a transmembrane C-terminal fragment, derived from a metalloproteinase cleavage after the E-cadherin ectodomain residue Pro700, approximately seven residues upstream of the transmembrane domain (i.e., amino acids

- 93 -

708-731 of SEQ ID NO: 34 or of the amino acid sequence provided as GenBank
Accession No. NP_004351). Metalloproteinase cleavage of the N-terminus of full-length
E-cadherin produces a 38 kDa fragment (E-Cad/CTF1) that binds both β -catenin and
PS1. Full-length E-cadherin and E-Cad/CTF1 are found only in the membrane and
5 cytoskeletal (Triton X-100-insoluble) fraction. Cleavage by PS1/ γ -secretase defines the
N-terminal region of a 33 kDa fragment (E-Cad/CTF2 or E-Cad intracellular carboxyl
domain (ICD)) that binds only β -catenin. A PS1/ γ -secretase cleavage of E-cadherin has
been shown between residues Leu731 and Arg732 (SEQ ID NO: 34 or the amino acid
sequence provided as GenBank Accession No. NP_004351) at the interface of the
10 membrane with the cytoplasm (Marambaud *et al.* 2002, *EMBO J.* 21(8):1948-56). E-Cad
ICD localizes in the membrane and in the soluble cytosol. Cleavage of E-cadherin by
caspase-3 between residues 750 and 751 has also been reported (Steinhusen *et al.* (2001)
J. Biol. Chem., 276:4972-4980). The PS1/ γ -secretase cleavage dissociates E-cadherins,
 β -catenin and α -catenin from the cytoskeleton, thus promoting disassembly of the E-
15 cadherin-catenin adhesion complex. Furthermore, this cleavage releases the cytoplasmic
E-cadherin intracellular carboxyl domain (ICD) to the cytosol and increases the levels of
soluble β - and α catenins. Thus, the PS1/ γ -secretase system stimulates disassembly of
the E-cadherin-catenin complex and increases the cytosolic pool of β -catenin, a key
regulator of the Wnt signaling pathway involved in cell proliferation.

20 In a method for assessing presenilin activity provided herein, the level of one or
more fragments of E-cadherin and/or the composition of E-cadherin is determined for a
sample for which presenilin activity is being assessed (examples of which are described
herein). E-cadherin can be added to the sample or, if the sample is a cell sample, E-
cadherin can be expressed endogenously and/or heterologously by the cell. In a
25 particular embodiment, the method includes assessing presenilin activity of a cell by
evaluating the level and/or presence or absence of a fragment from a C-terminal portion
of E-cadherin in a cell lysate.

To assess presenilin activity in these methods, the processing of E-cadherin is
evaluated. In evaluating E-cadherin processing, the composition of E-cadherin can be
30 evaluated. The composition of E-cadherin refers to the make-up of any E-cadherin that is

- 94 -

present anywhere in the analysis mixture, including the assay medium in which the analysis is being performed, an extracellular medium, or a cell membrane, lysate or extract. Thus, in one embodiment, the structure of any E-cadherin present can be evaluated to, for example, determine whether E-cadherin is intact or has been processed
5 and appears as a fragment or fragments of sizes smaller than the intact E-cadherin molecule. In evaluating E-cadherin processing, the levels and/or presence or absence of one or more E-cadherin fragments can be determined.

In particular, the E-cadherin composition is evaluated to determine if any fragment(s) indicative of presenilin-dependent cleavage (or altered presenilin-dependent
10 cleavage) of E-cadherin are present and/or the level of any such fragment(s). A presenilin-dependent cleavage described herein occurs within the C-terminal portion of E-cadherin. Thus, a presenilin-dependent cleavage of E-cadherin can be one that occurs in the C-terminal portion of E-cadherin at a position C-terminal to amino acid position 731 of SEQ ID NO: 34 (or of the amino acid sequence provided as GenBank Accession
15 No. NP_004351). The presenilin-dependent cleavage of E-cadherin can be one that occurs within the sequence of the last approximately 151, 150, 100, 50, 25, or less amino acids of E-cadherin. The presenilin-dependent cleavage can be one that occurs C-terminal to the extracellular portion of E-cadherin (i.e., C-terminal to amino acid 707 of SEQ ID NO: 34 or of the amino acid sequence provided as GenBank Accession No.
20 NP_004351). The presenilin-dependent cleavage of E-cadherin can be one that occurs near or within the region of the E-cadherin protein that extends from a point located intracellularly and adjacent to the cytoplasmic face of the cell membrane to a point located within the cell membrane. Such a presenilin-dependent cleavage of E-cadherin can be one that generates a soluble intracellular peptide containing the extreme C-
25 terminus of E-cadherin and a membrane-associated peptide containing amino acid sequence of the transmembrane region of E-cadherin, particularly the more C-terminal region of the transmembrane segment of E-cadherin. Any E-cadherin fragments generated by such presenilin-dependent activities would have a molecular weight that is less than that of the E-Cad/CTF1 fragment produced by metalloproteinase cleavage of
30 the N-terminus of full-length E-cadherin (E-Cad/CTF1 molecular weight is

- 95 -

approximately 38 kDa). Also, because caspase-3 can cleave a portion of the fragment produced by presenilin dependent cleavage, the molecular weight of such a fragment may be further reduced. Thus, an E-cadherin fragment generated by a presenilin-dependent cleavage can have a molecular weight that is, for example, less than about 40 kD, 35 kD, 5 30 kD, 25 kD, 20 kD, 15 kD or 10 kD or less. In a particular embodiment, an E-cadherin fragment generated by a presenilin-dependent cleavage has a molecular weight of less than about 35 kD or that is about 33 kD. An E-cadherin fragment generated by a presenilin-dependent cleavage can be one that can be detected by a reagent that binds to or recognizes an amino acid sequence from a C-terminal portion of E-cadherin.

10 As described herein above, E-cadherin is also cleaved by activities that are not presenilin dependent. Specifically, in one cleavage that is not a presenilin-dependent/ γ -secretase activity, full-length E-cadherin is cleaved by a metalloproteinase at the cell surface which results in "shedding" from the cell surface of a portion of the extracellular segment of E-cadherin (i.e., amino acids N-terminal of amino acid 701 of SEQ ID NO: 15 34 or of the amino acid sequence provided as GenBank Accession No. NP_004351). A fragment such as this that does not result from a presenilin-dependent cleavage is not alone indicative of presenilin activity.

In particular embodiments E-cadherin processing by PS1/ γ -secretase can be determined by measuring the levels of the E-cadherin ICD peptide and/or the E-cadherin 20 CTF1 peptide. In addition, the level, presence or absence of a Notch fragment that occurs in the absence of presenilin-dependent cleavage of Notch can be determined. For example, inhibition of the PS1/ γ -secretase processing of E-cadherin may result in the accumulation of the CTF1 peptide and/or a decrease in the level of the ICD peptide. E-cadherin peptide levels can be measured by any method known to those of skill in the art 25 for measuring protein level or by any method described herein. For example, levels of E-cadherin peptides may be measured by immunoassay using anti-E-Cad/CTF1 or anti-E-Cad ICD antibodies. Antibodies for use in such immunoassays can be obtained by the methods described herein or known to those of skill in the art such as those described by Marambaud *et al.* (*EMBO J.* (2002) 21(8):1948-56)..

30 4. Erb-B4 assay

- 96 -

Erb-B4 is a type I membrane receptor tyrosine kinase, which belongs to the epidermal growth receptor family and mediates response to multiple growth factors, including neuregulins. Erb-B4 has been implicated in many important biological and pathological processes, such as cardiovascular, mammary gland, and neuronal development, as well as malignancy and heart disease. The amino acid sequence of the ~180 kDa full-length Erb-B4 polypeptide is provided in SEQ ID NO: 36, and DNA encoding the polypeptide is provided in SEQ ID NO: 35. Constitutive ectodomain shedding of full-length Erb-B4 by a metalloprotease yields an ~80 kDa membrane-associated C-terminal fragment (B4-CTF) and a ~120 kDa ectodomain N-terminal fragment that is released into the extracellular medium. B4-CTF is further cleaved by a presenilin dependent γ -secretase releasing the soluble intracellular domain of Erb-B4 ICD which translocates to the nucleus and may participate in activation of gene transcription. The Erb-B4 ICD is believed to be ~80 kDa and contain a tyrosine kinase domain. Cleavage has been shown to occur at conserved residue Val673 on the C-terminal side of the transmembrane domain (residues 649-675 of amino acid SEQ ID NO: 36). This cleavage site is topologically similar to the γ -secretase cleavage site in Notch and cleavage of APP at conserved residue Val49.

In a method for assessing presenilin activity provided herein, the level of one or more fragments of Erb-B4 and/or the composition of Erb-B4 is determined for a sample for which presenilin activity is being assessed. Examples of a sample for which presenilin activity is being assessed are described herein. Erb-B4 can be added to the sample or can be expressed endogenously and/or heterologously by a cell in the sample. In a particular embodiment, the method includes assessing presenilin activity of a cell by evaluating the level and/or presence or absence of a fragment from a C-terminal portion of Erb-B4 in a cell lysate.

To assess presenilin activity in these methods, the processing of Erb-B4 is evaluated. In evaluating Erb-B4 processing, the composition of Erb-B4 can be evaluated. The composition of Erb-B4 refers to the make-up of any Erb-B4 that is present anywhere in the analysis mixture, including the assay medium in which the analysis is being performed, an extracellular medium, or a cell membrane, lysate or extract. Thus,

- 97 -

in one embodiment, the structure of any Erb-B4 present can be evaluated to, for example, determine whether Erb-B4 is intact or has been processed and appears as a fragment or fragments of sizes smaller than the intact Erb-B4 molecule. In evaluating Erb-B4 processing, the levels and/or presence or absence of one or more Erb-B4 fragments can
5 be determined.

The Erb-B4 composition can be evaluated to determine if any fragment(s) indicative of presenilin-dependent cleavage (or altered presenilin-dependent cleavage) of Erb-B4 are present and/or the level of any such fragment(s). A presenilin-dependent cleavage described herein occurs within the C-terminal portion of Erb-B4. Thus, a
10 presenilin-dependent cleavage of Erb-B4 can be one that occurs in the C-terminal portion of Erb-B4 at a position C-terminal to Val673 of SEQ ID NO: 36 (or of the amino acid sequence provided as GenBank Accession No. AAB59446). The presenilin-dependent cleavage of Erb-B4 can be one that occurs within the sequence of the last approximately 650, 600, 550, 500, 450, 400, 350, 300, 250, 200, 150, 100, 50, 25, or less amino acids of
15 Erb-B4. The presenilin-dependent cleavage can be one that occurs C-terminal to the extracellular portion of Erb-B4 (i.e., C-terminal to amino acid 648 of SEQ ID NO: 36 or of the amino acid sequence provided as GenBank Accession No. AAB59446). The presenilin-dependent cleavage of Erb-B4 can be one that occurs near or within the region of the Erb-B4 protein that extends from a point located intracellularly and adjacent to the
20 cytoplasmic face of the cell membrane to a point located within the cell membrane. Such a presenilin-dependent cleavage of Erb-B4 can be one that generates a soluble intracellular peptide containing the extreme C-terminus of Erb-B4 and a membrane-associated peptide containing amino acid sequence of the transmembrane region of Erb-B4, particularly the more C-terminal region of the transmembrane segment of Erb-B4.
25 Any Erb-B4 fragments generated by such presenilin-dependent activities would have a molecular weight that is less than that of the ~180 kDa full-length Erb-B4 polypeptide minus the ~120 kDa ectodomain N-terminal fragment that is released into the extracellular medium upon metalloproteinase cleavage. Thus, an E-cadherin fragment generated by a presenilin-dependent cleavage can have a molecular weight that is, for
30 example, less than about 100 kD, 90 kD, 80 kD, 70 kD, 60 kD, 50 kD, 40 kD, 30 kD, 20

- 98 -

kD, 15 kD or 10 kD or less. In a particular embodiment, an Erb-B4 fragment generated by a presenilin-dependent cleavage has a molecular weight of less than about 90 kD or that is about 80 kD. An Erb-B4 fragment generated by a presenilin-dependent cleavage can be one that can be detected by a reagent that binds to or recognizes an amino acid
5 sequence from a C-terminal portion of Erb-b4.

As described herein above, Erb-B4 is also cleaved by activities that are not presenilin dependent. Specifically, in one cleavage that is not a presenilin-dependent/ γ -secretase activity, full-length Erb-B4 is cleaved by a metalloproteinase at the cell surface which results in "shedding" from the cell surface of a portion of the extracellular segment
10 of E-cadherin (i.e., amino acids N-terminal of amino acid 648 of SEQ ID NO: 36 or of the amino acid sequence provided as GenBank Accession No. AAB59446). A fragment such as this that does not result from a presenilin-dependent cleavage generally is not alone indicative of presenilin activity.

In particular embodiments, Erb-B4 processing by PS1/ γ -secretase can be assessed
15 by determining the levels and/or presence or absence of the Erb-B4 ICD peptide and/or the Erb-B4 membrane-associated peptide. In addition, the level, presence or absence of an Erb-B4 fragment that occurs in the absence of presenilin-dependent cleavage of Notch can be determined. Erb-B4 peptide levels can be measured by any method known to those of skill in the art for measuring protein level or by any method described herein. In
20 a particular embodiment of the method, Erb-B4 peptide levels are measured by immunoassay. Anti-Erb-B4 peptide antibodies for use in such immunoassays can be obtained by the methods described herein or known to those of skill in the art. For example, polyclonal antibodies to the carboxyl terminus (residues 1291-1308) can be purchased (Santa Cruz Biotechnology, Inc.). Other antibodies to Erb-B4 peptides have
25 also been described (see, e.g., Ni, *et al.*, (2001) *Science* 294:2179-2181).

D. Methods of Identifying or Screening for Agents that Modulate A β Levels

Methods, and compositions for use therein, are provided for identifying or screening for agents that modulate the levels of one or more A β peptides in a sample. The sample may be any sample, such as described herein, and may be reflective of, e.g.,
30 cellular and/or extracellular A β levels. In a particular embodiment, the methods can be

- 99 -

- used to identify agents that modulate the levels of A β 42, including cellular and/or extracellular A β 42. In another embodiment, the methods can be used to identify an agent that selectively modulates the level of one or more A β peptides, such as, for example, A β 42, including cellular and/or extracellular peptides. For example, in one embodiment,
- 5 the method includes a step of identifying an agent that selectively modulates the level of one or two A β peptides relative to one or more other A β peptides. In a particular embodiment, an agent that selectively modulates the levels of A β 42 only or of A β 42 and A β 39 only, relative to other A β peptides (including, *e.g.*, A β 40, A β 38 and/or A β 43), is identified.
- 10 In another embodiment, the methods can be used to identify agents that modulate A β peptide levels (and, in particular, A β 42 levels) without substantially affecting (or with limited, minimal or inconsequential effect on) compositions, mechanisms, processes and/or activities that are not significantly involved in the generation, degradation and/or clearance of one or more A β peptides. In a particular embodiment, the method involves
- 15 a step of identifying an agent that modulates the levels (including cellular and/or extracellular) of one or more A β peptides without substantially altering the cleavage of a presenilin substrate, or portion thereof, that is not APP. In a further embodiment, the presenilin substrate is LRP. Included among the agents that can be identified using the methods provided herein are agents that modulate A β levels, for example, by modulating
- 20 compositions (*e.g.*, proteases and proteins, such as proteins on which protease activities depend, including presenilins), mechanisms and/or activities involved in A β peptide formation, degradation and/or clearance in cells and/or extracellular medium without substantially affecting (or with only limited, minimal or inconsequential effect on) compositions, mechanisms and/or activities that are not significantly involved in A β
- 25 peptide formation and persistence.

Agents identified by the methods provided herein have a variety of uses. For example, such agents can be used in elucidating the particular elements and pathways involved in A β peptide formation, degradation and clearance in cells. Such agents may be used to assess proteolytic processing in cells and to characterize enzyme and protein

30 interactions that facilitate and/or inhibit such processing. Proteolytic processing events

- 100 -

include, but are not limited to, those involved in the production and/or degradation of A β peptides. For example, agents identified by the methods may be used to identify and/or characterize regulatory molecules including, but not limited to, proteases that produce or degrade A β peptides and proteins involved in the activation or inhibition of such

5 proteases. In addition, because release of A β peptides is a normal event in virtually every cell, the agents identified herein can be used to further characterize the role of such peptides in biochemical pathways and/or normal cellular processes. The agents identified by the methods provided herein may also serve as candidate agents for the treatment and/or prevention of disorders and diseases characterized by and/or involving

10 inappropriate levels or misregulation of A β . Such diseases and disorders include any disease or disorder involving misregulation of A β production, clearance, and/or degradation. Exemplary disease and disorders include neurodegenerative diseases and disorders, such as, but not limited to, Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease, amyotrophic lateral sclerosis (ALS), Down's syndrome,

15 Hereditary Cerebral Hemorrhage with Amyloidosis-Dutch Type (HCHWA-D), and advanced aging of the brain. Such agents can provide therapeutic and/or preventative benefit with limited-to-no potential side effects that can result from non-specific modulation of A β peptide processing.

The methods provided herein for identifying or screening for agents that modulate

20 A β levels can be used to identify agents that modulate cell and/or cellular membrane (i.e., referred to herein as cellular) A β levels and/or extracellular A β levels. In general, the methods include steps of contacting a sample containing amyloid precursor protein (APP), and/or portion(s) thereof (e.g., one or more A β peptides), with a test agent and identifying an agent that alters the A β peptide-producing cleavage of the APP, the

25 processing of the APP, the processing of A β and/or the levels of one or more A β peptides in the sample.

The step of identifying an agent that alters the A β peptide-producing cleavage or processing of the APP (and/or portion(s) thereof), the processing of A β and/or the levels of one or more A β peptides in the sample can be carried out in a number of ways. In

30 general, the identification step can involve a comparison of the cleavage or processing of

- 101 -

APP (and/or portion(s) thereof), processing of A β and/or the A β levels of a sample that has been contacted with the test agent (i.e., test sample) and of a sample that has not been contacted with the test agent (i.e., control sample). If the A β -producing cleavage or processing of APP (and/or portion(s) thereof), the processing of A β and/or the A β levels of the test and control samples differ, then the agent is identified as one that modulates the level of one or more A β peptides. The control sample can be the same physical sample as the test sample or a different sample. When the control and test samples are the same, the control is the sample in the absence of test agent. Assessing the cleavage and processing of APP (and/or portion(s) thereof), the processing of A β , and the A β levels of a sample can be conducted in a number of ways such as described herein or known in the art. In a particular method for assessing the A β ₄₂ level of a sample, a monoclonal antibody provided herein that selectively binds A β ₄₂ relative to other A β peptides is used to in an immunoassay for the detection and/or quantitation of A β ₄₂.

The methods provided herein for identifying or screening for agents that modulate A β levels can also include identifying an agent that alters the A β peptide-producing cleavage or processing of the APP (and/or portion(s) thereof), the processing of A β and/or the levels of one or more A β peptides in the sample without substantially altering the cleavage of a presenilin substrate, or portion thereof, other than APP. In these methods, a sample containing a source of a presenilin substrate (or a portion thereof) other than APP is contacted with the test agent. The sample may be the same as the sample containing APP (and/or portion(s) thereof) or can be a different sample. The process of identifying an agent that alters the A β peptide-producing cleavage or processing of the APP (and/or portion(s) thereof), the processing of A β and/or the levels of one or more A β peptides in a sample can be carried out in a number of ways as described herein. In addition, the process of further identifying an agent that also does not substantially alter the cleavage of a presenilin substrate (other than APP), or portion thereof, can be carried out in a number of ways. In general, this process can involve a comparison of the presenilin-dependent cleavage and/or processing of a presenilin substrate (or portion thereof) other than APP and/or the levels of a peptide fragment or fragments of the presenilin substrate that is other than APP of a sample that has been

- 102 -

contacted with the test agent (i.e., test sample) and of a sample that has not been contacted with the test agent (i.e., control sample). If the presenilin-dependent cleavage or processing of the presenilin substrate that is other than APP and/or the substrate fragment(s) levels of the test and control samples do not differ substantially, then the agent is identified as one that alters the level of one or more A β peptides without substantially altering the cleavage of the presenilin substrate, or portion thereof, that is other than APP. The control sample can be the same physical sample as the test sample or a different sample. When the control and test samples are the same, the control is the sample in the absence of test agent.

10 **1. Samples for use in methods of identifying A β -modulating agents**

A sample that can be used in the methods of identifying an agent that modulates the A β levels can be any composition (e.g., a biological or physiological composition) that includes a source of APP, and/or portion(s) thereof, or a source of one or more A β peptides including, but not limited to, a cell, a cell extract or lysate, a cellular membrane and a cell-free medium. When the sample contains a source of APP, it generally also contains a source of enzymatic and/or other activity that provides for processing of APP, and, in particular, A β peptide-producing cleavage activity. When a sample is one for use in methods that include a step of identifying an agent that alters the processing, such as degradation, of A β , and thus contains a source of A β peptides, it generally also contains a source of enzymatic and/or other activity that provides for processing of A β (e.g., a catabolic activity that degrades A β).

a. APP or portion(s) thereof

The APP, and/or portion(s) thereof, provided by the source contained within the sample is generally any APP (and/or portion(s) thereof) that include(s) the A β peptide domains within its amino acid sequence. A β peptides include, but are not limited to, (1) a peptide that results from processing or cleavage of an APP and that is amyloidogenic, (2) one of the peptide constituents of β -amyloid plaques, (3) a fragment or portion of the 43-amino acid sequence set forth in SEQ ID NO: 4 and (4) a fragment or portion of a peptide as set forth in (1) or (2). A β peptides derived from proteolysis of APP, or degradation of A β , generally are typically 39 to 43 amino acids in length (see, e.g., SEQ

- 103 -

ID NO: 4 showing the 43-amino acid sequence of an A β peptide), depending on the carboxy-terminal end-point, which exhibits heterogeneity. However, A β peptides containing less than 39 amino acids, *e.g.*, A β 39, A β 38, A β 37 and A β 34, also may occur. A β peptides include those that begin at position 672 of APP770 (see SEQ ID NO: 2).

- 5 Isoforms of APP that contain an A β domain include APP770, APP751, APP714, APP695, L-APP752, L-APP733, L-APP696 and L-APP697. APP can be an APP of any species. In particular embodiments, the APP is a mammalian APP, such as, for example, a rodent or human APP.

- 10 In methods of identifying or screening for agents that modulate A β levels that include a step of identifying an agent that alters the A β peptide-producing cleavage of APP, the sample can contain a source of APP that can be cleaved or modified to yield one or more A β peptides. In methods that include a step of identifying an agent that alters the processing, such as degradation, of A β , the sample generally contains a source of A β peptides. Such a source can be, for example, synthetic, recombinant or isolated A β
- 15 peptides, or a source of APP that can be cleaved or modified to yield one or more A β peptides. In methods that include a step of identifying an agent that alters the processing of APP, the sample can contain a source of APP that can undergo processing. In methods that include a step of identifying an agent that alters the level of one or more A β peptides, the sample generally contains a source of A β peptides. Such a source can be,
- 20 for example, synthetic, recombinant or isolated A β peptides, or a source of APP that can be cleaved or modified to yield one or more A β peptides.

- Sources of APP, or a portion thereof, include, but are not limited to: a cell that expresses endogenous or heterologous APP; a cell that expresses a recombinant portion(s) or fragment(s) of APP; lysates, extracts, or membrane fractions of any such
- 25 cells; APP, or a portion thereof, that is isolated from such cells; and synthetic APP protein or synthetic proteins that represent a portion of APP.

- Sources of A β peptides include, but are not limited to: a cell that expresses endogenous or heterologous APP and enzymatic activities that cleave APP to yield A β peptides (*e.g.*, β - and γ -secretases); a cell that expresses recombinant A β peptides;
- 30 lysates, extracts, or membrane fractions of any such cells; A β peptides that are isolated

- 104 -

from such cells; synthetic or isolated APP that is degraded to yield A β peptides; and synthetic A β peptides.

Compositions, and methods of making compositions, that are sources of APP, portion(s) thereof, and A β peptides are described herein and known in the art. For example, cells that endogenously express APP and/or A β peptides are known in the art as are nucleic acids encoding APP (or portion(s) thereof) and/or A β peptides (see, *e.g.*, SEQ ID NOs: 1, 3, 27 and 29) that can be used to express the encoded proteins in cells. Methods of preparing lysates, extracts and membrane fractions of such cells are also described herein and known in the art, as are synthetic methods for generating proteins and peptides and preparatory methods of isolating proteins and peptides.

b. Sources of activities that provide for processing of APP and/or A β peptides

Sources of activities that provide for cleavage or processing of APP (or portion(s) thereof) and/or A β peptides include, but are not limited to: a cell that expresses endogenous or heterologous molecules that give rise to the activities; lysates, extracts, or membrane fractions of any such cells; molecules that give rise to the activities that are isolated from such cells; and synthetic molecules that give rise to the activities.

Molecules that can be involved in activities that provide for cleavage or processing of APP or A β include, but are not limited to, secretases, including α -, β - and γ -secretase, presenilins, including PS1 and PS2, insulin-degrading enzyme (IDE), neprilysin, plasmin, uPA/tPA, endothelin converting enzyme-1, and matrix metalloproteinase-9 (see, *e.g.*, Selkoe (2001) *Neuron* 32:177-180; Vekrellis *et al.* (2000) *J. Neurosci.* 20:1657-1665; Iwata *et al.* (2000) *Nat. Med.* 6:143-150; Carson and Turner (2002) *J. Neurochem* 81(1): 1-8; and Eckman *et al.* (2001) *J. Biol. Chem.* 276: 24540-24548). Such molecules can be from any species. In particular embodiments, the molecule is a mammalian molecule, such as, for example, a rodent or human molecule.

c. Conditions that enhance A β production

When a sample is one for use in methods that include a step of identifying an agent that alters the processing of A β and/or the levels of one or more A β peptides, it is generally desirable for the sample to contain a readily detectable amount of A β peptide.

- 105 -

To enhance A β production in a sample containing an A β -producing source, one or more modulatory molecules or compounds that provide for increased A β levels through increased A β production or decreased A β clearance can be included in the sample. For example, a modulatory molecule may function to activate β -secretase and/or γ -secretase
5 contained within the sample for increased processing of APP into A β peptides. Alternatively, a modulatory molecule may function to inhibit one or more A β -degrading proteases leading to decreased clearance of A β peptides. Exemplary modulatory molecules of this kind may include, but are not limited to serine protease inhibitors such as α 1-antichymotrypsin (Mucke *et al.* (2000) *An. J. Pathol.* 157: 2003-2010; Nilsson *et*
10 *al.* (2001) *J. Neurosci.* 21:1444-1451). In addition, the protease inhibitor thiorphan which is known to inhibit several proteases, has been shown to induce plaque formation in rats (Iwata *et al.* (2000) *Nat. Med.* 6: 143-150).

d. Medium

A sample medium can be any medium in which APP, portion(s) thereof, and/or
15 A β peptides can exist. Examples of sample medium include, but are not limited to, cells, cell lysates, extracts and membranes, and cell-free medium.

(1) Cells

(a) General features of cells

Although any cell may be used in the methods, cells that are particularly suitable
20 are those that exhibit APP and/or A β peptide synthesis and processing and/or those in which A β levels and/or processing may readily be assessed. If a cell has an APP processing and/or cleavage activity but does not express APP (or expresses APP at only low or undetectable levels), nucleic acid encoding APP can be introduced into the cells, and vice versa. If a cell has an A β catabolic activity (i.e., an activity that degrades one or
25 more forms of A β) but does not express A β (or expresses only low levels of A β or only particular forms of A β), nucleic acid encoding one or more A β peptides can be introduced into the cells, and vice versa. Cells that express enzymatic and/or other activities involved in APP and/or A β processing can also be used in conjunction with another or separate source of APP and/or A β peptides in the sample. Thus, transfected or

- 106 -

recombinant cells, as well as cells that endogenously express desired proteins and/or activities, can be used in the methods of identifying agents that modulate A β levels.

In particular examples, cells used in samples for the methods of identifying agents that modulate A β levels are eukaryotic cells. In a further example, the cells can be mammalian cells. Mammalian cells include, but are not limited to, rodent (*e.g.*, mouse, rat and hamster), primate, monkey, dog, bovine, rabbit and human cells. In particular embodiments of the methods, the sample includes a mammalian cell, such as, for example, a rodent or human cell, that expresses endogenous and/or heterologous APP (or a portion(s) thereof) and/or A β , and the activity or activities for processing and cleavage of APP and/or A β . Cells may also be cells of *in vivo* or *in vivo*-derived samples, including body fluids, such as but not limited to, serum, blood, saliva, cerebral spinal fluid, synovial fluid and interstitial fluids, urine, sweat and other such fluids and secretions.

Another feature of cells that are particularly suitable for use in the screening and identification methods is amenability to transfection/transformation with heterologous nucleic acid and amenability to gene expression alteration. A number of techniques for the introduction of heterologous nucleic acid into cells and for altering gene expression in cells are known in the art and described herein. The relative ease with which these techniques may be applied to a cell to effect recombinant expression of a heterologous nucleic acid, or reduction, alteration or elimination of one or more genes in the cell is a consideration in selection of cells for use in the methods provided herein. Amenability to gene expression alteration and analysis of A β may be considerations, for example, when screening agents in AD model systems (as described herein).

(b) Cells that exhibit APP and/or A β production

Exemplary cells that exhibit APP and/or A β production include, but are not limited to, primary cell cultures, typically neuronal cell cultures. Primary cells from any organism that exhibits APP and/or A β production and/or processing may be used. Examples include mixed fetal guinea pig brain cells (Beck (2000) *Neuroscience* 95:243-254). Primary cell cultures are harvested from a mammal and cultured using standard techniques and include cortical neural cells, microglia, glia, astrocytes, and the like.

- 107 -

Briefly, neural tissue including but not limited to the brain of a mammal expressing or diagnosed with AD symptoms is harvested, and optionally subjected to enzymatic digestion to ease the separation of cells. The cells can be mechanically separated as well. Cells can also be enriched by type or characteristic using standard techniques. Primary
5 culture cells, typically neural tissue, can be induced to express A β in response to growth factors, cytokines, hormones, or transcription pathway activators. Thus, suitable cells include cells capable of expressing A β in response to an A β -inducing agent. An A β -inducing agent means any substance that causes and/or enhances the expression of APP or A β and includes, but is not limited, to growth factors including but not limited to TGF,
10 TGF- β , PDGF, and EGF; cytokines, hormones or a combination thereof.

Totipotent, pluripotent, or other cells that are not terminally differentiated can be induced to express neuronal characteristics including the production of A β peptides. Exemplary non-terminally differentiated cells include embryonic stem cells, adult stem cells, mesenchymal stem cells, bone marrow stem cells, adipose tissue stem cells, and
15 neuronal stem cells. These non-terminally differentiated cells can be induced to express A β when exposed to growth factors, cytokines, morphogenetic factors, or tissue specific inducing media. Thus, cells that can be used in the methods of identifying or screening for agents that modulate A β levels include non-terminally differentiated cells induced to express A β . The non-terminally differentiated cells can be of any lineage, endoderm,
20 mesoderm, or ectoderm or a combination thereof.

Other cells that express APP and/or A β include immortalized cell lines transfected or transformed with exogenous nucleic acids encoding APP, A β , a precursor, or fragment thereof. For example, US Patent. No. 5,538,845, incorporated by reference, describes the transfection of chinese hamster ovary (CHO) cells and 293 human
25 embryonic kidney (HEK) cell line, ATCC accession number CRL-1573, with cDNA encoding the 695, 751, and 770 amino acid isoforms of APP. Mouse neuroblastoma cells (*e.g.*, N2A cells; ATCC accession number CCL-131) are another example of cells that can be transfected with nucleic acid encoding APP, a portion(s) thereof or A β . Any of these cells can be cotransfected, if necessary, with vectors comprising nucleic acid
30 sequences encoding β -secretase, γ -secretase and/or presenilin for the processing of APP

- 108 -

to generate A β peptides.

Additionally, SH-SY5Y cells, a human neuroblastoma cell line that secretes A β into the culture medium without β APP transfection, can be used. This cell line is available from ECACC European Collection of Cell Cultures, CAMR Centre for Applied
5 Microbiology & Research Porton Down, Salisbury, Wiltshire (UK) SP4 0JG UK under accession number 94030304.

Cells transfected with nucleic acid constructs can express APP and/or A β peptides using standard expression vectors. Expression can be, for example, constitutive or induced.

10 (2) **Cell lysates, extracts and membranes and cell-free medium**

Biological compositions that can be used as samples in the methods of identifying or screening for agents that modulate A β levels include, but are not limited to, purified or partially purified enzyme preparations, conditioned medium from cultured cells, cellular
15 extracts and cell lysates. Cell lysates can be generated using methods described herein (see, e.g., Example 8) and/or known in the art. For example, cell lysates can be prepared from cells able to process APP into A β and/or able to catabolize A β . Alternatively, appropriate APP processing or catabolic enzymes may be incubated with cell lysates devoid of such activity.

20 (3) ***In vivo* systems**

In addition, as described below, *in vivo* organism systems can also be used in methods of identifying A β -modulating agents. The organism can be one that produces endogenous APP and/or A β peptides and processing and cleavage activities or a transgenic organism (non-human) that has been generated to express heterologous APP
25 and/or A β peptides and/or processing and cleavage activities. Organisms include, but are not limited to, mammals (e.g., rodents) salmon (Maldonado *et al.* (2000) *Brain Res.* 858:237-251), and invertebrate animals, for example, *Drosophila* and *C. elegans* (see, e.g., Link (2001) *Mech. Ageing Dev.* 122:1639-1649).

For example, in methods of identifying agents that modulate A β levels, an
30 organism can be contacted with a test agent and the levels of A β in any sample from the

- 109 -

organism, *e.g.*, tissue, plasma, CSF and brain, can be compared between treated and untreated organisms. Plasma and CSF can be obtained from an organism using standard methods. For example, plasma can be obtained from blood by centrifugation, CSF can be isolated using standard methods, and brain tissue can be obtained from sacrificed organisms. The organism can be contacted with a test agent in various ways. For example, the test agent can be dissolved in a suitable vehicle and administered orally or by injection. The test agent also can be administered as a component of drinking water or feed.

2. Identification of agents that modulate A β levels

Cellular and extracellular A β levels, and the degree of A β accumulation, are dependent on A β production, through APP cleavage and processing, as well as on A β catabolism, degradation and clearance. A method for identifying or screening for agents that modulate A β levels can include steps of contacting a sample containing APP (and/or portion(s) thereof) with a test agent and identifying an agent that alters any one or more aspects of A β production and/or A β catabolism. Thus, the method can include a step of identifying an agent that alters the A β peptide-producing cleavage of APP, the processing of APP, the processing of A β and/or the levels of one or more A β peptides in a sample.

a. Assessment of A β peptide-producing cleavage of APP and APP processing

Any of the methods for identifying or screening for agents that modulate A β levels can include a step of assessing A β peptide-producing cleavage of APP and APP processing of a sample. For samples that contain a source of APP and of an APP-processing activity, a variety of methods are provided for assessment of A β peptide-producing cleavage of APP and APP processing. In a particular embodiment measurement of A β levels of the sample (as described in detail below) can provide a method for assessing A β peptide-producing cleavage of APP and APP processing. In other embodiments, measurement of APP fragments levels in a sample other than A β can be used as a means for assessing A β peptide-producing cleavage of APP and APP processing. In other embodiments, measurement of the activity of one or more enzymes in the sample can be used to assess A β peptide-producing cleavage of APP and APP

- 110 -

processing. The one or more enzymes are enzymes that participate in either the amyloidogenic or non-amyloidogenic APP cleavage pathways.

As described herein, APP can undergo proteolytic processing via two pathways: an amyloidogenic pathway and a non-amyloidogenic pathway. In the non-amyloidogenic pathway, cleavage of APP by α -secretase occurs at position 16 within the A β domain releasing the large N-terminal secreted ectodomain of APP ending at the α -secretase cleavage site (sAPP α) and a non-amyloidogenic C-terminal fragment of about 10 kD (C83; the 83-amino acid carboxyl tail of APP). Because α -secretase cleaves within the A β domain, this cleavage precludes A β formation. Rather, the C-terminal fragment of APP generated by α -secretase cleavage is subsequently cleaved by γ -secretase within the predicted transmembrane domain to generate a 22-24 residue non-amyloidogenic peptide fragment termed p3.

Alternatively, in the amyloidogenic pathway, cleavage of APP by β -secretase (BACE) occurs at the beginning of the A β domain defining the amino terminus of the A β peptide. This cleavage generates a shorter soluble N-terminus, APP β , as well as an amyloidogenic C-terminal fragment (C99), the 99-amino acid C-terminal fragment that contains the transmembrane and cytoplasmic domains of APP. Further cleavage of this C-terminal fragment by γ -secretase, a presenilin-dependent enzyme, generates A β .

The activity of β -secretase versus α -secretase and, thus, the proportion of APP processed by these enzymes will affect the amount of A β produced. Swedish APP mutations have been mapped to the β -secretase cleavage site in APP and favor β secretase cleavage of APP. Thus, cells expressing these mutations secrete increased amounts of A β and decreased amounts of p3 as compared with cells expressing wild-type APP. In contrast to the Swedish mutation, which increases β -secretase cleavage, activation of protein kinase C (PKC) by phorbol 12-myristate 13-acetate (PMA) has been shown to favor α -secretase cleavage at the expense of β -secretase cleavage (Skovronsky *et al.*, (2000) *J. Bio. Chem.* 275: 2568-2575) indicating that PKC-regulated α -secretase competes directly with β -secretase for cleavage of APP. Furthermore, changes in levels of APP-CTFs have been shown to mirror changes seen in sAPP β and sAPP α (e.g.,

- 111 -

increased levels of A β or decreased levels of p3 can be indicated by an increase in sAPP β or by a similar decrease in sAPP α)

Since β -secretase activity may be limited by the availability of APP, then increased cleavage of APP by other secretases could decrease β -secretase cleavage of APP and hence A β production. Also, by the same reasoning, decreased cleavage of APP by other secretases could increase β -secretase cleavage of APP leading to increased A β production. It can, therefore, generally be assumed that an alteration in the non-amyloidogenic pathway will result in a similar but opposite alteration in the amyloidogenic pathway. Thus, agents that modulate enzymes or the regulation of enzymes in either the amyloidogenic or non-amyloidogenic pathway can modulate levels of A β . As a result, peptide-producing cleavage of APP and APP processing may be assessed by measuring the activity of such enzymes. Assessment of the activity of such enzymes can provide information about peptide-producing cleavage of APP and APP fragment production pattern (i.e., the types and amounts of APP peptide fragments produced by APP fragment production enzymes). Alternatively, assessment of peptide fragments (particularly non-A β peptide fragments) produced in both pathways (APP fragment production patterns) can provide information about the activities of enzymes in the pathways and peptide producing cleavage of APP. In a particular embodiment, A β peptide-producing cleavage of APP can be assessed by monitoring the activity of enzymes and/or the cleavage of APP by enzymes of the non-amyloidogenic pathway, specifically α -secretase activity and/or the levels of fragments generated by α -secretase activity including sAPP α , C83 and p3 peptide fragments. Likewise, agents that alter the A β peptide-producing cleavage of APP and APP processing may be screened for by monitoring enzyme activities and/or fragmentation patterns in the presence and absence of test agents.

b. Assessment of A β processing

Any of the methods for identifying or screening for agents that modulate A β levels can include a step of assessing A β processing of a sample. For samples that contain a source of APP and an APP-processing activity, methods such as those described above can be provided for assessment of A β processing. For samples that contain

- 112 -

a source of A β and of an A β degradation activity, a variety of methods are provided for assessment of A β processing. In a particular embodiment measurement of A β levels of the sample (as described in detail below) can provide a method for assessing A β processing. In other embodiments, measurement of the activity of one or more

5 degradation and/or clearance pathways and/or degradation fragment patterns in the sample can be used to assess A β processing. The one or more pathways include, but are not limited to, proteolytic degradation, receptor-mediated clearance, non-receptor-mediated clearance, and/or aggregation/fibrillogenesis. Defects in pathways for A β degradation and clearance can lead to an alteration in the levels of A β and, therefore,

10 could underlie some or many cases of amyloidosis and other neurodegenerative disease such familial and sporadic AD as well as other diseases and disorders characterized by misregulation of A β . A β processing may, therefore, be assessed by monitoring enzyme activities involved in the degradation and clearance of A β . In addition, fragmentation patterns of A β produced upon cleavage by degradative enzymes may be used to assess

15 A β processing. There are numerous proteases in the brain that could potentially participate in A β turnover, and there is evidence that several enzymes may contribute to the degradation of A β peptides in brain tissue including insulin-degrading enzyme (IDE), neprilysin, plasmin, uPA/tPA, endothelin converting enzyme-1, and matrix metalloproteinase-9 (Selkoe J. (2001) *Neuron* 32:177-180). Similarly, agents that alter

20 A β processing may be screened for by monitoring the activity of one or more enzymes involved in the degradation and/or clearance of A β and/or fragmentation patterns of resulting degradation products in the presence and absence of test agents.

c. Assessment of A β levels

Any of the methods for identifying or screening for agents that modulate A β

25 levels can include a step of assessing A β levels of a sample. For samples that contain a source of APP and of an APP-processing activity, an assessment of A β levels of the sample can provide a method for assessing A β peptide-producing cleavage of APP and for assessing APP processing. For samples that contain a source of A β peptides and of A β catabolic activity, an assessment of A β levels of the sample can provide a method for

30 assessing processing of A β . For samples that contain a source of APP, A β , APP-

- 113 -

processing activity, and a source of A β catabolic activity, an assessment of A β levels of the sample can provide a method for assessing the overall balance of A β peptide-producing cleavage of APP, APP processing and A β processing.

In assessing the A β levels of a sample, the total A β (i.e., all forms of A β) level
5 can be assessed in an indiscriminant determination of the A β level of a sample, or the level of one or more specific forms of A β can be assessed. In one embodiment of the methods, the level of A β 42, A β 40, A β 39 and/or A β 38 is assessed. In a particular embodiment, the level of A β 42 is assessed.

Methods and compositions for indiscriminant assessment of total A β levels and
10 for selective assessment of a particular A β peptide are provided herein. In a method provided herein for indiscriminant assessment of total A β levels, the sample, or portion thereof, is contacted with an antibody that binds to forms of A β that contain amino acids 1-12 of SEQ ID NO: 4. Also provided is an antibody that binds to forms of A β that
15 contain amino acids 1-12 of SEQ ID NO: 4. In a method provided herein for the selective assessment of A β 42 levels, the sample, or portion thereof, is contacted with an antibody that selectively binds to A β 42 (*e.g.*, the sequence of amino acids 1-42 of SEQ ID NO: 4) relative to other forms of A β . Also provided is an antibody, and portions thereof, that selectively bind to A β 42 relative to other forms of A β .

The A β levels of a sample or any portion(s) thereof may be assessed in the
20 methods. For example, if the sample is a cell-free medium or culture medium, the A β levels of the medium can be assessed. If the sample is a cell sample, the A β levels of the extracellular medium (*e.g.*, secreted A β) of the sample and/or the cellular (*e.g.*, intracellular and/or membrane-associated A β) A β levels can be assessed. To assess the cellular A β levels, lysates, extracts, and/or membranes of the cells can be analyzed for
25 A β protein. If the sample is an organism, then the cellular, tissue, and/or secreted A β levels can be assessed. For example, secreted A β levels could be assessed in fluids of the organism, such as, for example, any bodily fluids. Levels of secreted A β may be monitored, for example, by the methods described in Example 6. Preparation of whole cell lysates and membrane fractions are well known to those of skill in the art. Cell
30 lysates may be obtained for instance by the method described in Example 8 for the

identification of LRP-CTFs.

(1) Procedures for assessing A β levels

Assessment of the A β level of a sample or portion(s) thereof can be conducted using methods described herein or any method known in the art for detecting the presence of and/or measuring the level or amount of a peptide or protein in a sample. For example, immunological detection techniques employing binding substances such as antibodies, antibody fragments, recombinant antibodies, and the like, can be used. Detection of A β peptide can be carried out using any standard antibody-based assays. Exemplary immunoassays are described in detail, for example, in *Antibodies: A Laboratory Manual*, Harlow and Lane (Eds.), Cold Spring Harbor Laboratory Press, 1988. Representative examples of such assays include, for example, concurrent immunoelectrophoresis, radioimmunoassay, immunoprecipitation, western hybridization, and enzyme-linked immunosorbent assays (ELISA), inhibition or competition assay, and sandwich assay. Suitable immunological methods employing a single antibody are also contemplated, for example, radioimmunoassay using an antibody specific for a particular form of A β , or single antibody ELISA methods.

Mass spectrometry and electrophoretic analysis of at least partially purified A β peptides are also techniques that can be used to detect and quantitate A β . In addition, the levels of different forms of A β can be quantified using known methods such as, for example, using internal standards and/or calibration curves generated by performing the assay with known amounts of standards.

(2) Immunological methods for A β detection

A β peptides, which can differ by only a single amino acid, can be fairly similar in molecular weight. Therefore, methods, such as immunological methods, that are based in detecting properties of A β peptides that can be more distinctive than molecular weight (at least when using standard and relatively inexpensive laboratory reagents and equipment) can be well-suited for assessing the level of a particular A β peptide. Methods and compositions for use in immunoassays for A β peptides in general are described herein.

Compositions and methods for detecting A β peptides that contain the sequence of

- 115 -

amino acids 1-12 of SEQ ID NO: 4, or a portion of this sequence, are provided herein. The compositions and methods are based on the generation of antibodies against a peptide having the amino acid sequence of amino acids 1-12 of SEQ ID NO: 4. In a particular embodiment, the antibody is B436, or a fragment thereof (see Examples 2 and 5 4). Because most A β peptides contain such a sequence, these compositions and methods are particularly useful in assessing the total A β content of a sample and in detecting most forms of A β .

More particularly, compositions and methods for detecting A β 42 or assessing the A β 42 content of a sample are provided herein. The compositions and methods are based 10 on the development of an antibody that selectively binds A β 42 relative to other A β peptides. In a particular embodiment, the antibody is A387, or a fragment thereof (see Examples 1 and 4).

(a) Antibody preparation

Antibodies specific for A β may be prepared against a suitable antigen or hapten 15 comprising the desired target epitope. The target epitope may include any number of amino acids within any portion of an A β amino acid sequence. SEQ ID NO: 4 provides the amino acid sequence of a 43-amino acid form of a human A β (A β 43). Shorter forms of human A β peptides include, but are not limited to, those having the amino acid sequence of amino acids 1-42, 1-40, 1-39, 1-38, 1-37 and 1-34 of SEQ ID NO: 4. 20 Typically, the target epitope will include at least 2 contiguous residues and may include more than 6 contiguous residues within any portion of the A β amino acid sequence. The target epitope may include a sequence of amino acids from the amino terminus typically any of amino acids 1-13, the junction region typically containing any of the amino acids residues 13-26 and the carboxy terminus typically containing any of the amino acid 25 residues 33-42.

A target epitope composed of such peptide fragments may be prepared, for example, from mammals such as humans, monkeys, rats and mice by methods which are known to those of skill in the art, and may also be purified natural samples which are commercially available. Partial peptides can be obtained by hydrolyzing longer forms of 30 A β successively from the N-terminus and/or the C-terminus with exoproteases such as

- 116 -

aminopeptidase and carboxypeptidase or mixtures thereof or various endopeptidases or mixtures thereof.

Synthetic peptides may be prepared by methods known in the art including solid phase synthesis methods and liquid phase synthesis methods. Examples of such

5 synthesis methods include methods described in Merrifield, (1963) *J. Am. Chem. Soc.* 85:2149-2156; Bodanszky and Ondetti, *Peptide Synthesis*, Interscience Publishers, New York (1966); and Schroder and Lubke, *The Peptide*, Academic Press, New York, (1965).

For example, when the A β peptides are synthesized by solid methods, any resins known in the art as insoluble resins (such as chloromethyl resins and 4-

10 oxymethylphenylacetamidomethyl resins) are used for a successive condensation of protected amino acids to the C-terminal sides of the A β synthetic peptides according to usual methods. The protective groups are removed by hydrogen fluoride treatment, followed by purification by methods which are known in the art, such as high performance liquid chromatography. Thus, the desired A β peptides can be obtained.

15 N-protected amino acids can be produced by the methods of protecting the α -amino-groups with Boc groups; further, for example, the hydroxyl groups of serine and threonine with Bzl groups; the ω -carboxylic acid groups of glutamic acid and aspartic acid with OBzl groups; the ϵ -amino group of lysine with a Cl-Z group; the guanido group of arginine with a Tos group; and the imidazole group of histidine with a Bom group.

20 Once a sufficient quantity of peptide hapten has been obtained, it may be conjugated to a suitable immunogenic carrier. Natural polymer carriers can be used as immunogenic carriers and include, for example, albumin, thyroglobulin, hemoglobin, keyhole limpet hemocyanin, or other suitable protein carriers, as generally described in Hudson and Hay, *Practical Immunology*, Blackwell Scientific Publications, Oxford,
25 Chapter 1.3, 1980. Examples of synthetic polymer carriers that can be used include various latexes of polymers or copolymers such as amino acid polymers, styrene polymers, acrylic polymers, vinyl polymers and propylene polymers. An exemplary immunogenic carrier utilized in the Examples provided herein is ovalbumin. Since A β peptides aggregate easily, insolubilized A β haptens can also be directly immunized
30 without the use of a carrier.

- 117 -

In addition, various condensing agents can be used for coupling of the haptens and the carriers. Examples of the condensation agents include diazonium compounds such as bis-diazotized benzidine which crosslinks tyrosine, histidine and tryptophan; dialdehyde compounds such as glutaraldehyde which crosslinks amino groups together; 5 diisocyanate compounds such as toluene-2,4-diisocyanate; dimaleimide compounds such as N,N'-o-phenylenedimaleimide which crosslinks thiol groups together; maleimide active ester compounds which crosslink amino groups and thiol groups; and carbodiimide compounds crosslinking amino groups and carboxyl groups. When amino groups are crosslinked together, there is another way in which an active ester reagent (for 10 example, SPDP) having a dithiopyridyl group is reacted with one amino acid, followed by reduction to introduce a thiol group, whereas a maleimide group is introduced into the other amino group by the use of a maleimide active ester reagent, and then, both can be reacted with each other. Examples of such methods for crosslinking haptens with immunogenic carriers can be found, for example, in U.S. Patent Nos. 4,140,662 and 15 4,486,344.

Haptens can be used alone or together with carriers and diluents to produce antibodies specific for the desired epitope by in vitro or in vivo techniques. In vitro techniques involve exposure of lymphocytes to the immunogens, while in vivo techniques require the injection of the immunogens into a suitable vertebrate host. 20 Suitable vertebrate hosts are non-human, including, for example, monkeys, dogs, guinea pigs, mice, rats, rabbits, sheep, goats, and chickens. Immunogens are delivered to the animal according to a predetermined schedule, and the animals are periodically bled, with successive bleeds having improved titer and specificity. The immunogens can be delivered to any antibody-producible site, for example, by intramuscular, intraperitoneal, 25 subcutaneous and intravenous injections. Adjuvant may also be employed to enhance antibody production. Adjuvants may provide for sustained release of the injected immunogen, serve as a vehicle to help deliver the immunogen to the spleen and/or lymph nodes, and/or work to activate the various cells involved in the immune response, either directly or indirectly. Adjuvants may include, for example, Freund's Complete Adjuvant, 30 Freund's Incomplete Adjuvant, Montanide ISA Adjuvants (Seppic, Paris, France), Ribi's

- 118 -

Adjuvants (Ribi Immuno Chem Research, Inc., Hamilton, MT), Hunter's TiterMax (CytRx Corp., Norcross, GA), Aluminum Salt Adjuvants, nitrocellulose-adsorbed protein, encapsulated antigens (such as liposome-entrapped antigen, nondegradable ethylene-vinyl acetate copolymer (EVAc)-entrapped antigen, and degradable polymer-entrapped antigen), and Gerbu Adjuvant (Gerbu Biotechnik GmbH, Gaiberg, Germany/C-C Biotech, Poway, CA).

Antibody producing cells can be obtained by hyperimmunizing a host animal, such as a mouse, with the desired immunogen by the methods described herein. The host is then killed, usually several days after the final immunization, the spleen and/or lymph nodes cells collected, and the cells immortalized resulting in anti-A β monoclonal antibody-producing hybridomas. Immortalization may be carried out by any method known to those of skill in the art or provided herein. Methods of immortalization may include, for example, fusion with a myeloma cell fusion partner (Kohler and Milstein (1975) *Nature* 256:495-497), EBV transformation, and transformation with bare DNA, e.g., oncogenes or retroviruses, or any other method which provides for stable maintenance of the cell line and production of monoclonal antibodies such as those described in *Antibodies: A Laboratory Manual*, Harlow and Lane, eds., Cold Spring Harbor Laboratory, 1988. An exemplary immortalization method utilized in the Examples provided herein is the fusion of mouse spleen cells with mouse thymocytes.

Hybridomas can then be cloned and screened for avidity. Antibody avidity is the functional affinity or combining strength of an antibody with its antigen and is related to both the affinity of the reaction between the epitopes and paratopes, and the valences or recognition sites of the antibody and antigen. Avidity can be viewed as the total binding strength of all of an antibody's binding sites together. Affinity of an antibody reflects the goodness of fit of an antigenic determinant to a single antigen-binding site and is independent of the number of sites. Methods of assaying for antibody binding affinity are well known to those of skill in the art. Affinity or binding strength is generally expressed as the affinity constant (K). The affinity constant, alternatively called an association constant (K_a), can be determined by measuring the concentration of free antigen required to fill half of the antigen-binding sites on the antibody. The reciprocal

- 119 -

of the antigen concentration that produces half-maximal binding is equal to the affinity constant of the antibody for the antigen. The affinity constant can be determined by measuring the association or dissociation constant for an antibody. Association and dissociation constants can be determined, for example, using a competition ELISA.

5 The degree of recognition of an antibody for an antigen is related to the selectivity (or specificity) of an antibody. Selectivity is considered a measure of the functional ability of an antibody to discriminate between the target antigen and other, chemically similar structures. Methods of assaying for antibody binding selectivity are well known to those of skill in the art. Selectivity can be determined, for example, by comparing the
10 binding affinity of the antibody for the target antigen with the binding affinity of the antibody for other chemically similar molecules. Positive clones producing antibodies with high affinity and selectivity for specific A β peptides of interest can thus be chosen.

 The desired monoclonal antibodies can be produced by injecting the hybridoma cells selected for their ability to produce high avidity antibodies into mice or by growing
15 them in culture. With in vivo production, hybridoma cells are injected intraperitoneally into syngeneic animals, such as, for example, BALB/c mice or SCID mice, and ascites fluid obtained and purified. In addition, a primer or adjuvant may be used, such as, for example, pristane (2,6,10,14-tetramethyl pentadecane) or incomplete Freund's adjuvant to suppress the immune system so that the growth of the hybridoma cells is not strongly
20 impaired, and to prohibit toxic irritation which may lead to peritonitis and the secretion of serous fluid. Purification may be carried out using standard antibody purification techniques, such as, for example, affinity chromatography using Protein A or Protein G.

i. A β 42-selective antibody

 Particular embodiments of the methods provided herein for identifying or
25 screening for agents that modulate A β levels include a step of identifying an agent that modulates the level of A β 42 in a sample. In one embodiment, the step involves identifying an agent that selectively modulates the level of A β 42 in a sample relative to A β 40 and/or increasing the level of A β 39. Thus, the practice of some of the methods provided herein involves the ability to detect a particular species of A β , such as A β 42,
30 and to distinguish it from other species (e.g., from other A β forms that do not contain the

- 120 -

"42" carboxy terminus, such as A β 40). Antibodies and fragments thereof selective or specific for A β 42 are provided herein. Also provided are isolated antibodies selective or specific for A β 42. Further provided are amino acid sequences and proteins that portions of the antibodies. Also provided are isolated proteins that are portions of the antibodies. In a particular embodiment, the antibody is a mouse antibody. In a particular embodiment, the antibody is a monoclonal antibody, such as, for example, a mouse monoclonal antibody. In one embodiment, the A β 42-selective antibody is one generated against a peptide based on a mammalian A β amino acid sequence, including, for example, a human A β amino acid sequence. In a particular embodiment, the A β 42-selective antibody is an IgG. In one embodiment, the antibody type is IgG2a kappa.

The A β 42-selective antibodies provided herein bind A β 42 with minimal to no binding of other A β forms, *e.g.*, A β 1-40, A β 1-11, 1-28, 1-38, and 1-39). In a particular embodiment, the A β 42-selective antibody has at least 100-fold, 200-fold, 300-fold, 400-fold, 500-fold, 600-fold, 700-fold, 800-fold, 900-fold, 1000-fold or more specificity or selectivity for A β 42 relative to other forms of A β , and, in particular A β 40. In one embodiment, the A β 42-selective antibody has at least about 1000-fold specificity or selectivity for A β 42 relative to A β 40. The antibodies selective for A β 42 provided herein have a high affinity for binding to A β 42. In a particular embodiment, the antibody has an affinity constant for binding to A β 42 of at least about 10^5 l/mol, 2×10^5 l/mol, 3×10^5 l/mol, 4×10^5 l/mol, 5×10^5 l/mol, 6×10^5 l/mol, 7×10^5 l/mol, 8×10^5 l/mol, 9×10^5 l/mol, 10^6 l/mol, 2×10^6 l/mol, 3×10^6 l/mol or 4×10^6 l/mol or more. In one embodiment, the antibody has an affinity constant for binding to A β 42 of at least about 4×10^6 l/mol. In a particular embodiment, the A β 42-selective antibody has an affinity constant for binding to A β 42 of at least about 4×10^6 l/mol and at least about 1000-fold specificity or selectivity for A β 42 relative to A β 40.

In one embodiment, an antibody or portion or fragment thereof provided herein contains a sequence of amino acids as set forth in SEQ ID NO: 12. In a particular embodiment, an antibody or portion or fragment thereof contains a sequence of amino acids set forth as amino acids 1-107 or 1-95 of SEQ ID NO: 12. In another embodiment, an antibody or portion or fragment thereof contains a light chain variable region

- 121 -

containing the sequence of amino acids 1-95 of SEQ ID NO: 12. In a further embodiment, an antibody or portion or fragment thereof contains a light chain variable region containing the sequence of amino acids selected from 1-50, 1-60, 1-70, 1-80, 1-90, 1-91, 1-92, 1-93, or 1-94 of SEQ ID NO: 12. In a particular embodiment, the light chain

5 is a kappa light chain. In another embodiment, the antibody or portion or fragment thereof that contains a sequence of amino acids set forth as amino acids 1-95 of SEQ ID NO: 12 further contains a joining (J) region. In a particular embodiment, the J region is a J_{kappa} region. The J region can be one from any species, including but not limited to mammals, such as, for example, a primate, a rodent, or a human. In one embodiment, the

10 J region contains a sequence of amino acids set forth as amino acids 96-107 as set forth in SEQ ID NO: 12. In one embodiment, an antibody or portion or fragment thereof contains a sequence of amino acids set forth as amino acids 1-95 of SEQ ID NO: 12 and a sequence of amino acids of a constant (C) region, such as, for example, a light chain C region. The C region can be one from any species, including but not limited to

15 mammals, such as, for example, a primate, a rodent, or a human. In a particular embodiment, the C region is a C_{kappa} region. For example, the C region can contain the sequence of amino acids set forth in SEQ ID NOs: 63, 65 or 81. In a particular embodiment the antibody or fragment thereof contains a sequence of amino acids as set forth in SEQ ID NO: 97.

20 In one embodiment, an antibody or portion or fragment thereof provided herein contains a sequence of amino acids set forth in SEQ ID NO: 14. In a particular embodiment, an antibody or portion or fragment thereof contains a sequence of amino acids set forth as amino acids 1-118 or 1-97 of SEQ ID NO: 14. In one embodiment, an antibody or portion or fragment thereof contains a heavy chain variable region containing

25 the sequence of amino acids 1-97 of SEQ ID NO: 14. In a further embodiment, an antibody or portion or fragment thereof contains a heavy chain variable region containing the sequence of amino acids selected from 1-50, 1-60, 1-70, 1-80, 1-90, 1-91, 1-92, 1-93, 1-94, 1-95, or 1-96 of SEQ ID NO: 14. In a particular embodiment, the heavy chain is a γ heavy chain. In one embodiment, the antibody is an IgG_{2a}. In another embodiment, the

30 antibody or portion or fragment thereof that contains a sequence of amino acids set forth

- 122 -

as amino acids 1-97 of SEQ ID NO: 14 further contains a diversity and joining ("DJ") region. The DJ region can be one from any species, including but not limited to mammals, such as, for example, a primate, a rodent, or a human. In a particular embodiment, the DJ region is a heavy chain DJ region, such as a DJ_γ region. In one
5 embodiment, the DJ region contains a sequence of amino acids set forth as amino acids 98-118 as set forth in SEQ ID NO: 14. In one embodiment, an antibody or portion or fragment thereof contains a sequence of amino acids set forth as amino acids 1-97 of SEQ ID NO: 14 and a sequence of amino acids of a constant (C) region, such as, for example, a heavy chain C region. The C region can be one from any species, including
10 but not limited to mammals, such as, for example, a primate, a rodent, or a human. In a particular embodiment, the C region is a C_γ region. For example, the C region can contain the sequence of amino acids set forth in SEQ ID NO: 69, 71, 83, 85 or 87. In a particular embodiment the antibody or fragment thereof contains a sequence of amino acids as set forth in SEQ ID NO: 98.

15 In one embodiment, an antibody or portion or fragment thereof contains the sequence of amino acids set forth in SEQ ID NO: 12 (or amino acids 1-107 or 1-95 of SEQ ID NO: 12) and the sequence of amino acids set forth in SEQ ID NO: 14 (or amino acids 1-118 or 1-97 of SEQ ID NO: 14). In another embodiment, an antibody or portion or fragment thereof contains the sequence of amino acids set forth in SEQ ID NO: 12 (or
20 amino acids 1-107 or 1-95 of SEQ ID NO: 12) and the sequence of amino acids set forth in SEQ ID NO: 14 (or amino acids 1-118 or 1-97 of SEQ ID NO: 14) and further contains amino acid sequence of one or more J and/or DJ regions. For example, the J region can be a light and/or heavy chain J region. The J and/or DJ region(s) can be from any species, including, but not limited to mammals, such as, for example, primates,
25 rodents and humans. Exemplary J regions include, but are not limited to, a J_{kappa} region (*e.g.*, such as one containing a sequence of amino acids 96-107 as set forth in SEQ ID NO: 12) and/or a heavy chain DJ region, such as a DJ_γ region (*e.g.*, such as one containing a sequence of amino acids 98-118 as set forth in SEQ ID NO: 14). Other exemplary J regions include, but are not limited to, a light chain J region (*e.g.*, such as
30 one containing a sequence of amino acids set forth in SEQ ID NO: 46, 48, 50, 52, 54, 55,

- 123 -

57, 59, 61, 73, 75, 77, or 79 and/or a heavy chain J region (*e.g.*, such as one containing a sequence of amino acids set forth in SEQ ID NO: 67, 89, or 91). In another embodiment, an antibody or portion or fragment thereof contains the sequence of amino acids set forth in SEQ ID NO: 12 (or amino acids 1-107 or 1-95 of SEQ ID NO: 12) and the sequence of amino acids set forth in SEQ ID NO: 14 (or amino acids 1-118 or 1-97 of SEQ ID NO: 14) and further contains amino acid sequence of one or more constant regions. For example, the constant region can be a light and/or heavy chain constant region. The C region(s) can be from any species, including, but not limited to mammals, such as, for example, primates, rodents and humans. Exemplary constant regions include, but are not limited to, a C_κ region. Exemplary light chain constant regions may contain a sequence of amino acids set forth in SEQ ID NO: 63, 65 or 81. Exemplary constant regions may also include, but are not limited to, a C_γ region. Exemplary heavy chain constant regions may contain a sequence of amino acids set forth in SEQ ID NO: 69, 71, 83, 85 or 87. In a particular embodiment of any of the antibodies, the antibody or portion or fragment thereof is an IgG_{2a} type.

Also provided are derivatives and modified immunoglobulins that have the capacity to bind to Aβ. In a particular embodiment, such molecules include fragments, such as Fab' or Fab'2 produced, for example, by the proteolytic cleavage of the mAb. Such molecules may also include single-chain immunoglobulins producible, for example, via recombinant means, such as Fv, scFv. Portions or fragments of antibodies include fragments that contain at least a portion of the antigen-binding region of the antibody. The portion of the antigen-binding region can be one that binds to the same antigenic determinant as the antibody with an affinity of at least about 1%, 5%, 10%, 15%, 20%, 25%, 50%, 60%, 70%, 75%, 80%, 90% or 100% of the affinity of the entire antibody. In particular embodiments, such fragments can be combined with one another (*e.g.*, to form a diabody) or with other antibody fragments or receptor ligands to form "chimeric" binding molecules. Significantly, such chimeric molecules may contain substituents capable of binding to different epitopes of the same molecule (*e.g.*, two different Aβ epitopes). Whole antibodies molecules are large proteins, ~150 kDa in size, made up of four chains, two heavy chains (~50 kDa each), and two light chains (~25 kDa each). The

- 124 -

domains responsible for targeting specifically foreign entities is called the Fv domains. The Fv domain contains a portion of a heavy chain domain (HFv) and a light domain (LFv). Fv's are not produced by the body but can be engineered. An scFv fragment is an entity very similar to the Fv fragment, except the heavy and light chains are connected
5 via a linker sequence. A dimer of scFv fragments is called a diabody. Fab fragments contain portions of heavy and light domains that are chemically linked. Fab fragments can be prepared from the parent antibody, by simple enzymatic hydrolysis. Thus, a "portion or fragment" of antibody refers to any of these aforementioned antibody fragments as well as to any fragment or portion of an antibody that retains an at least
10 100-fold, 200-fold, 300-fold, 400-fold, 500-fold up to 1000-fold selectivity for A β 42 relative to other A β peptides, and particularly relative to A β 40.

Also provided herein are nucleic acids encoding an antibody or a portion or fragment thereof. Further provided are isolated nucleic acids containing nucleotide sequences encoding portions of the antibodies. In a particular embodiment, the antibody
15 is a mouse antibody. In one embodiment, a nucleic acid contains a sequence of nucleotides that encodes the amino acid sequence set forth in SEQ ID NO: 12. In a particular embodiment, a nucleic acid contains a sequence of nucleotides that encodes the amino acid sequence set forth as amino acids 1-107 or 1-95 of SEQ ID NO: 12. In one embodiment, a nucleic acid contains a sequence of nucleotides that encodes a light chain
20 variable region containing the sequence of amino acids 1-95 of SEQ ID NO: 12. In a further embodiment, a nucleic acid contains a sequence of nucleotides that encodes a light chain variable region containing the sequence of amino acids selected from 1-50, 1-60, 1-70, 1-80, 1-90, 1-91, 1-92, 1-93, or 1-94 of SEQ ID NO: 12. In a particular embodiment, the light chain is a kappa chain. In another embodiment, a nucleic acid
25 contains a sequence of nucleotides that encodes the sequence of amino acids 1-95 of SEQ ID NO: 12 and a J region. The J region can be from any species, including but not limited to mammals, such as, for example, primates, rodents and humans. For example, the J can contain the sequence of amino acids set forth in SEQ ID NO: 46, 48, 50, 52, 54, 55, 57, 59, 61, 73, 75, 77 or 79. In a particular embodiment, the J region contains a
30 sequence of amino acids 96-107 as set forth in SEQ ID NO: 12. In another embodiment,

- 125 -

a nucleic acid contains a sequence of nucleotides that encodes the sequence of amino acids 1-95 of SEQ ID NO: 12 and a constant (C) region. The C region can be from any species, including but not limited to mammals, such as, for example, primates, rodents and humans. In particular embodiments, the C region is a light chain C region. For
5 example, the C region can be a kappa light chain constant sequence. In one example, the C region can contain the sequence of amino acids set forth in SEQ ID NO: 63, 65 or 81. In another embodiment, a nucleic acid can further contain, in addition to a sequence of nucleotides encoding a C region, a sequence of nucleotides encoding a variable region such as those described herein above. In a particular embodiment, a nucleic acid
10 provided herein contains the sequence of nucleotides set forth in SEQ ID NO: 11 or the sequence of nucleotides 1-285 set forth in SEQ ID NO: 11.

In another embodiment, a nucleic acid contains a sequence of nucleotides that encodes the amino acid sequence set forth in SEQ ID NO: 14. In a particular embodiment, a nucleic acid contains a sequence of nucleotides that encodes the amino
15 acid sequence set forth as amino acids 1-118 or 1-97 of SEQ ID NO: 14. In one embodiment, a nucleic acid contains a sequence of nucleotides that encodes a heavy chain variable region containing the sequence of amino acids 1-97 of SEQ ID NO: 14. In a further embodiment, a nucleic acid contains a sequence of nucleotides that encodes a heavy chain variable region containing the sequence of amino acids selected from 1-50,
20 1-60, 1-70, 1-80, 1-90, 1-91, 1-92, 1-93, 1-94, 1-95, or 1-96 of SEQ ID NO: 14. In a particular embodiment, the heavy chain is an IgG_{2a} heavy chain. In another embodiment, a nucleic acid contains a sequence of nucleotides that encodes the sequence of amino acids 1-97 of SEQ ID NO: 14 and a DJ region. The DJ region can be from any species, including but not limited to mammals, such as, for example, primates, rodents and
25 humans. For example, the DJ can contain the sequence of amino acids set forth in SEQ ID NO: 67, 89 or 91. In a particular embodiment, the DJ region contains a sequence of amino acids corresponding to 98 through 118 as set forth in SEQ ID NO: 14. In another embodiment, a nucleic acid contains a sequence of nucleotides that encodes the sequence of amino acids 1-97 of SEQ ID NO: 14 and one or more a constant (C) regions. The C
30 region can be from any species, including but not limited to mammals, such as, for

- 126 -

example, primates, rodents and humans. In particular embodiments, the C region is a heavy chain C region. For example, the C region can be a heavy chain C_γ region. For example, the C region can contain the sequence of amino acids set forth in SEQ ID NO: 69, 71, 83, 85 or 87. In a particular example, the C region can be an IgG_{2a} heavy chain constant sequence. In another embodiment, a nucleic acid can further contain, in addition to a sequence of nucleotides encoding a C region, a sequence of nucleotides encoding a variable region such as those described herein above. In a particular embodiment, a nucleic acid provided herein contains the sequence of nucleotides set forth in SEQ ID NO: 13 or the sequence of nucleotides 1-291 of SEQ ID NO: 13.

10 Nucleic acid constructs, including, for example, plasmids and expression vectors, are also provided herein. In one embodiment of a nucleic acid construct provided herein, the nucleic acid contains a sequence of nucleotides encoding the sequence of amino acids set forth in SEQ ID NO: 12 (or amino acids 1-107 or 1-95 of SEQ ID NO: 12) and the sequence of amino acids set forth in SEQ ID NO: 14 (or amino acids 1-118 or 1-97 of

15 SEQ ID NO: 14). In a another embodiment, a nucleic acid provided herein contains the sequence of nucleotides set forth in SEQ ID NO: 11 and SEQ ID NO: 13. In another embodiment, a nucleic acid provided herein contains the sequence of nucleotides set forth in SEQ ID NO: 11 and SEQ ID NO: 13 or a sequence of nucleotides encoding the sequence of amino acids set forth in SEQ ID NO: 12 (or amino acids 1-107 or 1-95 of

20 SEQ ID NO: 12) and a sequence of nucleotides encoding the sequence of amino acids set forth in SEQ ID NO: 14 (or amino acids 1-118 or 1-97 of SEQ ID NO: 14) and further contains one or more sequences of nucleotides encoding one or more of the following amino acid sequences: a J region, *e.g.*, a light or a heavy chain J region, including, for example, a kappa light chain J region and a γ heavy chain J region, and a C region, *e.g.*, a

25 light chain or heavy chain constant region, including, for example, a kappa light chain constant region, and a γ heavy chain C region, such as an IgG_{2a} heavy chain constant region. These regions can be from any species, including, but not limited to, mammals, such as, for example, primates, rodents and humans. Exemplary amino acid sequences of such regions can be any of those described herein above or known in the art.

30 Antibodies selective or specific for Aβ₄₂ can be made by immunizing an animal

- 127 -

(*e.g.*, a mouse) with a peptide that contains a sequence of amino acids within the sequence of A β 1-42 (such as human A β 1-42; see, *e.g.*, SEQ ID NO: 4 amino acids 1-42) that includes amino acids C-terminal to amino acid 40 of A β . In a particular example, to minimize the likelihood of cross-reactivity of a generated antibody with the predominant

5 A β 40 species, a minimal peptidyl sequence of C-MVGGVIA was used to immunize animals, which represents the A β 35-42 region (*e.g.*, amino acids 35-42 of A β ; see amino acids 35-42 of SEQ ID NO: 4). An N-terminal cysteine can be added for conjugation to an immunogenic carrier such as, for example, ovalbumin as described in Example 1. In a particular embodiment, an A β 42-selective antibody provided herein is the monoclonal

10 antibody A387 (described in detail in Examples 1 and 4). Antibody A387 demonstrates very high affinity for A β 42 with a measured affinity constant of $>4 \times 10^6$ l/mol. Furthermore, A387 has at least 1000-fold specificity for binding to A β 42 versus A β 40. Additionally, this antibody was shown to be highly selective for A β 42 versus other AB peptides. When tested by ELISA methods, the A387 antibody showed no reactivity to

15 A β 1-11, 1-28, 1-38, and 1-39 peptides. The exceptionally high affinity and selectivity of the A β 42-selective antibodies provided herein makes them a highly effective tool for detecting and quantitatively measuring A β 42 and distinguishing this form of A β from other A β forms. Additionally, the A β 42-selective antibodies provided herein are particularly useful for specifically assaying samples that contain detergents (such as

20 Triton X-100, CHAPS, SHAPSO, Tween-2, and the like) or metal chelators (EDTA, EGTA, and the like) for A β 42.

Antibodies provided herein can also be produced using recombinant DNA methods. For example, the recombinant production of immunoglobulin molecules, including humanized antibodies are described in U.S. Pat. Nos. 4,816,397 (Boss et al.),

25 4,816,567 (Cabilly et al.) U.K. patent GB 2,188,638 (Winter et al), and U.K. patent GB 2,209,757. Techniques for the recombinant expression of immunoglobulins, including humanized immunoglobulins, can also be found, among other places in Goeddel et al, Gene Expression Technology Methods in Enzymology Vol. 185 Academic Press (1991), and Borreback, Antibody Engineering, W. H. Freeman (1992). Additional information

30 concerning the generation, design and expression of recombinant antibodies can be found

- 128 -

in Mayforth, Designing Antibodies, Academic Press, San Diego (1993).

The host cell used to express the recombinant antibodies provided herein may be either a bacterial cell, such as *Escherichia coli*, or a eukaryotic cell, such as a chinese hamster ovary cell. The choice of expression vector is dependent upon the choice of host cell, and may be selected so as to have the desired expression and regulatory characteristics in the selected host cell. The general methods for construction of the vector, transfection of cells to produce the host cell, culture of cells to produce the antibody are all well known in the art. Likewise, once produced, the recombinant antibodies may be purified by standard procedures of the art, including cross-flow filtration, ammonium sulphate precipitation, affinity column chromatography, gel electrophoresis and the like.

Antibodies can be made by constructing a vector containing a nucleic acid encoding a V region. Exemplary V regions include any of those described herein. The V region can be fused with a J region. The J region can be, for example, a light chain J region or a heavy chain J region, including, for example, a kappa light chain J region and a γ heavy chain J region. These regions can be from any species, including, but not limited to, mammals, such as, for example, primates, rodents and humans. Exemplary J regions include, but are not limited to, a J_{kappa} region (*e.g.*, such as one containing a sequence of amino acids 96-107 as set forth in SEQ ID NO: 12 or a sequence of amino acids 101-112 as set forth in SEQ ID NO: 16) and/or a heavy chain DJ region, such as a DJ _{γ} region (*e.g.*, such as one containing a sequence of amino acids 98-118 as set forth in SEQ ID NO: 14 or a sequence of amino acids 99-114 as set forth in SEQ ID NO: 18). Other exemplary J regions include, but are not limited to, human and mouse light chain J regions (*e.g.*, such as the ones containing a sequence of amino acids set forth in SEQ ID NOS. 73, 75, 77 or 79, and SEQ ID NOS. 46, 48, 50, 52, 54, 55, 57, 59 or 61 respectively) and human and mouse heavy chain J region (*e.g.*, such as the ones containing a sequence of amino acids set forth in SEQ ID NOS. 89 or 91, and SEQ ID NO. 67 respectively). In constructing the vector the nucleic acid encoding the V and J regions can further be fused with nucleic acid encoding a C region. The C region can be, for example, a light chain C region or a heavy chain C region, including, for example, a

- 129 -

kappa light chain constant region, and a γ heavy chain C region, such as an IgG_{2a} heavy chain constant region. These regions can be from any species, including, but not limited to, mammals, such as, for example, primates, rodents and humans. For example, mouse and human light chain C regions may contain a sequence of nucleotides that encodes the amino acid sequence set forth in SEQ ID NO. 63 or 65 and SEQ ID NO 81, respectively.
5 Mouse and human heavy chain C regions may contain a sequence of nucleotides that encodes the amino acid sequence set forth in SEQ ID NO. 69 or 71 and SEQ ID NO 83, 85 or 87, respectively.

In certain embodiments, the recombinant antibodies provided herein may
10 comprise a complete antibody molecule having full length heavy and light chains, or any fragment thereof, such as the Fab or (Fab')₂ fragments, a heavy chain and light chain dimer, or any minimal fragment thereof such as a Fv, an SCA (single chain antibody), and the like, specific for the particular A β antigen molecule.

The term humanized immunoglobulin or humanized antibody refers to an
15 immunoglobulin comprising portions of immunoglobulins of different origin, wherein at least one portion is of human origin. Accordingly, provided herein are humanized immunoglobulins which bind to a mammalian A β peptide (e.g., human A β 42 or A β 40), said immunoglobulin comprising an antigen-binding region of nonhuman origin (e.g., rodent) and at least a portion of an immunoglobulin of human origin (e.g., a human
20 framework region, a human constant region or portion thereof). For example, the humanized antibody can comprise portions derived from an immunoglobulin of nonhuman origin with the requisite specificity, such as a mouse, and from immunoglobulin sequences of human origin (e.g., a chimeric immunoglobulin), joined together chemically by conventional techniques (e.g., synthetic) or prepared as a
25 contiguous polypeptide using genetic engineering techniques (e.g., DNA encoding the protein portions of the chimeric antibody can be expressed to produce a contiguous polypeptide chain).

Another example of the humanized immunoglobulins provided herein is an immunoglobulin containing one or more immunoglobulin chains comprising a CDR of
30 nonhuman origin (e.g., one or more CDRs derived from an antibody of nonhuman origin)

- 130 -

and a framework region derived from a light and/or heavy chain of human origin (e.g., CDR-grafted antibodies with or without framework changes). In one embodiment, the humanized immunoglobulins can compete with murine A387 or B436 monoclonal antibodies for binding to the respective human A β peptides. In a particular embodiment, the antigen-binding region of the humanized immunoglobulin (a) is derived from A387 monoclonal antibody (e.g., as in a humanized immunoglobulin comprising CDR1, CDR2 and CDR3 of the A387 light chain and CDR1, CDR2 and CDR3 of the A387 heavy chain) or (b) is derived from B436 monoclonal antibody (e.g., as in a humanized immunoglobulin comprising CDR1, CDR2 and CDR3 of the B436 light chain and CDR1, CDR2 and CDR3 of the B436 heavy chain). Chimeric or CDR-grafted single chain antibodies are also encompassed by the term humanized immunoglobulin.

As set forth above, such humanized immunoglobulins can be produced using synthetic and/or recombinant nucleic acids to prepare genes (e.g., cDNA) encoding the desired humanized chain. For example, nucleic acid (e.g., DNA) sequences coding for humanized variable regions can be constructed using PCR mutagenesis methods to alter DNA sequences encoding a human or humanized chain, such as a DNA template from a previously humanized variable region (see e.g., Kamman, M., et al., Nucl. Acids Res., 17: 5404 (1989)); Sato, K., et al., Cancer Research, 53: 851-856 (1993); Daugherty, B. L. et al., Nucleic Acids Res., 19(9): 2471-2476 (1991); and Lewis, A. P. and J. S. Crowe, Gene, 101: 297-302 (1991)). Using these or other suitable methods, variants can also be readily produced. In one embodiment, cloned variable regions can be mutagenized, and sequences encoding variants with the desired specificity can be selected (e.g., from a phage library; see e.g., Krebber et al., U.S. Pat. No. 5,514,548; Hoogenboom et al., WO 93/06213, published Apr. 1, 1993)).

In certain embodiments, the humanized antibodies provided herein may comprise a complete antibody molecule having full length heavy and light chains, or any fragment thereof, such as the Fab or (Fab')₂ fragments, a heavy chain and light chain dimer, or any minimal fragment thereof such as a Fv, an SCA (single chain antibody), and the like, specific for the particular A β antigen molecule.

In one embodiment, an antibody or portion or fragment thereof provided herein

- 131 -

contains a sequence of amino acids as set forth in SEQ ID NO: 12 and/or SEQ ID NO: 14 (or portions thereof such as amino acids 1-95 of SEQ ID NO: 12 and/or amino acids 1-97 of SEQ ID NO: 14) or modifications thereof that retain the antigen-binding properties of an antibody containing one or both of these sequences of amino acids. Such
5 modifications can be determined empirically and include, for example, conservative amino acid substitutions as well as deletions and additions of residues that do not substantially alter the antigen-binding properties. Determination of residues that do not substantially alter antigen binding properties can be accomplished empirically, such as by systematic replacement of each residue in the polypeptide with another amino acid, such
10 as alanine, serine or glycine, and testing of the resulting polypeptide for its ability to bind to the antigen compared to the unmodified polypeptide. Those that retain at least 1, 10, 25, or 50% of the binding affinity compared to the unmodified polypeptide or that have an affinity constant of at least 10^6 are identified. Also polypeptides that include a portion of SEQ ID NO: 12, 14, 16, or 18 and retain such ability and modification thereof are
15 included.

Also provided herein are methods for detecting A β 42 and/or measuring A β 42 levels or determining the A β 42 content of a sample. The methods use antibodies provided herein. In one embodiment, the method includes steps of contacting a sample with an antibody or portion or fragment thereof provided herein and determining if the
20 antibody (or portion or fragment thereof) forms any complexes with or binds to any molecules in the sample. The contacting can be performed under conditions whereby the antibody (or portion or fragment thereof) binds to or forms a complex with A β . In a particular embodiment, the antibody is selective for A β 42 relative to other forms of A β , including A β 1-11, 1-28, 1-38, 1-39 and 1-40. In one embodiment, the antibody is
25 selective for A β 42 relative to A β 40. In other embodiments, the antibody or portion or fragment thereof is any one of the compositions as set forth herein above or described anywhere herein, including the Examples.

Specific immunoassay-related techniques and procedures that may be used in the methods for detecting A β 42 and/or measuring A β 42 levels or determining the A β 42
30 content of a sample are described herein or known in the art. Any such procedures may

- 132 -

be employed in the methods. Exemplary formats include, but are not limited to, ELISA, sandwich assays, competitive immunoassays, radioimmunoassays, Western blots and indirect immunofluorescent assays. In a particular embodiment of the methods for detecting A β 42 and/or measuring A β 42 levels or determining the A β 42 content of a sample provided herein, an A β 42-selective antibody or portion or fragment thereof provided herein is contacted with the sample, and binding between the antibody (or portion or fragment thereof) and any protein or peptide in the sample is assessed in a sandwich assay, as described herein.

ii. A β 1-12 antibody

Antibodies that react substantially similarly to any A β peptide which contains an amino-terminal sequence substantially as set forth in the sequence of amino acids 1-12 of SEQ ID NO: 4 are also provided herein. Also provided are isolated proteins that are portions of the antibodies. Included among such antibodies are antibodies referred to herein as A β 1-12 antibodies. Such antibodies can be used, for example, in immunoassays to detect all forms of A β (total A β), or at least all forms of A β containing the amino-terminus as set forth in amino acids 1-12 of SEQ ID NO: 4. Such antibodies can also be used in conjunction with antibodies that are selective for a particular type or types of A β , *e.g.*, A β 42 (including A β 42-selective antibodies provided herein), for example, to determine the ratio of A β 42 to total A β in a sample. Antibodies that react substantially similarly to any A β peptide can also be used as capture or detection antibodies in conjunction with selective antibodies in sandwich immunoassays to detect a particular form of A β , *e.g.*, A β 42. Such methods using the A β 1-12 antibodies and A β 42-selective antibodies provided herein are described herein.

In one embodiment, an antibody or portion or fragment thereof provided herein contains a sequence of amino acids as set forth in SEQ ID NO: 16. In a particular embodiment, an antibody or portion or fragment thereof contains a sequence of amino acids set forth as amino acids 1-112 or 1 to 100 of SEQ ID NO: 16. In one embodiment, an antibody or portion or fragment thereof contains a light chain variable region containing the sequence of amino acids 1-100 of SEQ ID NO: 16. In a further embodiment, an antibody or portion or fragment thereof contains a light chain variable

- 133 -

region containing the sequence of amino acids selected from 1-50, 1-60, 1-70, 1-80, 1-90, 1-95, 1-96, 1-97, 1-98, or 1-99 of SEQ ID NO: 16. In a particular embodiment, the light chain is a kappa light chain. In another embodiment, the antibody or portion or fragment thereof that contains a sequence of amino acids set forth as amino acids 1-100 of SEQ ID NO: 16 further contains a joining (J) region. In a particular embodiment, the J region is a J_{kappa} region. The J region can be one from any species, including but not limited to mammals, such as, for example, a primate, a rodent, or a human. In one embodiment, the J region contains a sequence of amino acids set forth as amino acids 101-112 as set forth in SEQ ID NO: 16. In one embodiment, an antibody or portion or fragment thereof contains a sequence of amino acids set forth as amino acids 1-100 of SEQ ID NO: 16 and a sequence of amino acids of a constant (C) region, such as, for example, a light chain C region. The C region can be one from any species, including but not limited to mammals, such as, for example, a primate, a rodent, or a human. In a particular embodiment, the C region is a C_{kappa} region. For example, the C region can contain the sequence of amino acids set forth in SEQ ID NOs: 63, 65 or 81. In a particular embodiment the antibody or fragment thereof contains a sequence of amino acids as set forth in SEQ ID NO: 99.

In one embodiment, an antibody or portion or fragment thereof provided herein contains a sequence of amino acids set forth in SEQ ID NO: 18. In a particular embodiment, an antibody or portion or fragment thereof contains a sequence of amino acids set forth as amino acids 1-114 or 1-98 of SEQ ID NO: 18. In one embodiment, an antibody or portion or fragment thereof contains a heavy chain variable region containing the sequence of amino acids 1-98 of SEQ ID NO: 18. In a further embodiment, an antibody or portion or fragment thereof contains a heavy chain variable region containing the sequence of amino acids selected from 1-50, 1-60, 1-70, 1-80, 1-90, 1-91, 1-92, 1-93, 1-94, 1-95, 1-96, or 1-97 of SEQ ID NO: 18. In a particular embodiment, the heavy chain is a γ heavy chain. In one embodiment, the antibody is an IgG_{2a}. In another embodiment, the antibody or portion or fragment thereof that contains a sequence of amino acids set forth as amino acids 1-98 of SEQ ID NO: 18 further contains a diversity and joining (DJ) region. The DJ region can be one from any species, including but not

- 134 -

limited to mammals, such as, for example, a primate, a rodent, or a human. In a particular embodiment, the DJ region is a heavy chain DJ region, such as a DJ_γ region. In one embodiment, the DJ region contains a sequence of amino acids set forth as amino acids 99-114 as set forth in SEQ ID NO: 18. In one embodiment, an antibody or portion
5 or fragment thereof contains a sequence of amino acids set forth as amino acids 1-98 of SEQ ID NO: 18 and a sequence of amino acids of a constant (C) region, such as, for example, a heavy chain C region. The C region can be one from any species, including but not limited to mammals, such as, for example, a primate, a rodent, or a human. In a particular embodiment, the C region is a C_γ region. For example, the C region can
10 contain the sequence of amino acids set forth in SEQ ID NO: 69, 71, 83, 85 or 87. In a particular embodiment the antibody or fragment thereof contains a sequence of amino acids as set forth in SEQ ID NO: 100

In one embodiment, an antibody or portion or fragment thereof contains the sequence of amino acids set forth in SEQ ID NO: 16 (or amino acids 1-112 or 1-100 of
15 SEQ ID NO: 16) and the sequence of amino acids set forth in SEQ ID NO: 18 (or amino acids 1-114 or 1-98 of SEQ ID NO: 18). In another embodiment, an antibody or portion or fragment thereof contains the sequence of amino acids set forth in SEQ ID NO: 16 (or amino acids 1-112 or 1-100 of SEQ ID NO: 16) and the sequence of amino acids set forth in SEQ ID NO: 18 (or amino acids 1-114 or 1-98 of SEQ ID NO: 18) and further
20 contains amino acid sequence of one or more J regions. For example, the J region can be a light and/or heavy chain J region. The J and/or DJ region(s) can be from any species, including, but not limited to mammals, such as, for example, primates, rodents and humans. Exemplary J regions include, but are not limited to, a J_{kappa} region (*e.g.*, such as one containing a sequence of amino acids 101-112 as set forth in SEQ ID NO: 16) and/or
25 a heavy chain DJ region, such as a DJ_γ region (*e.g.*, such as one containing a sequence of amino acids 99-114 as set forth in SEQ ID NO: 18). Other exemplary J regions include, but are not limited to, a light chain J region (*e.g.*, such as one containing a sequence of amino acids set forth in SEQ ID NO: 46, 48, 50, 52, 54, 55, 57, 59, 61, 73, 75, 77, or 79) and/or a heavy chain J region (*e.g.*, such as one containing a sequence of amino acids set
30 forth in SEQ ID NO: 67, 89 or 91). In another embodiment, an antibody or portion or

- 135 -

fragment thereof contains the sequence of amino acids set forth in SEQ ID NO: 16 (or amino acids 1-112 or 1-100 of SEQ ID NO: 16) and the sequence of amino acids set forth in SEQ ID NO: 18 (or amino acids 1-114 or 1-98 of SEQ ID NO: 18) and further contains amino acid sequence of one or more constant regions. For example, the

5 constant region can be a light and/or heavy chain constant region. The C region(s) can be from any species, including, but not limited to mammals, such as, for example, primates, rodents and humans. Exemplary constant regions include, but are not limited to, a C_{kappa} region. Exemplary light chain constant regions may contain a sequence of amino acids set forth in SEQ ID NO: 63, 65 or 81. Exemplary constant regions may also include, but

10 are not limited to, a C_γ region. Exemplary heavy chain constant regions may contain a sequence of amino acids set forth in SEQ ID NO: 69, 71, 83, 85 or 87. In a particular embodiment of any of the antibodies, the antibody or portion or fragment thereof is an IgG_{2a} type.

In one embodiment, an antibody or portion or fragment thereof provided herein

15 contains a sequence of amino acids as set forth in SEQ ID NO: 16 and/or SEQ ID NO: 18 (or portions thereof such as amino acids 1-100 of SEQ ID NO: 16 and 1-98 of SEQ ID NO: 18) or modifications thereof that retain the antigen-binding properties of an antibody containing one or both of these sequences of amino acids. Such modifications can be determined empirically and include, for example, conservative amino acid

20 substitutions as well as deletions and additions of residues that do not substantially alter the antigen-binding properties. Determination of residues that do not substantially alter antigen binding properties can be accomplished empirically, such as by systematic replacement of each residue in the polypeptide with another amino acid, such as alanine, serine or glycine, and testing of the resulting polypeptide for its ability to bind to the

25 antigen compared to the unmodified polypeptide. Those that retain at least 1, 10, 25, or 50% of the binding affinity compared to the unmodified polypeptide or that have an affinity constant of at least 10⁶ are identified. Also polypeptides that include a portion of SEQ ID NO: 16 or 18 and retain such ability and modification thereof are included.

Also provided herein are nucleic acids encoding an antibody or a portion or

30 fragment thereof. Further provided are isolated nucleic acids containing nucleotide

- 136 -

sequences encoding portions of antibodies. In a particular embodiment, the antibody is a mouse antibody. In one embodiment, a nucleic acid contains a sequence of nucleotides that encodes the amino acid sequence set forth in SEQ ID NO: 16. In a particular embodiment, a nucleic acid contains a sequence of nucleotides that encodes the amino acid sequence set forth as amino acids 1-112 or 1-100 of SEQ ID NO: 16. In one
5 embodiment, a nucleic acid contains a sequence of nucleotides that encodes a light chain variable region containing the sequence of amino acids 1-100 of SEQ ID NO: 16. In a further embodiment, a nucleic acid contains a sequence of nucleotides that encodes a light chain variable region containing the sequence of amino acids selected from 1-50, 1-
10 60, 1-70, 1-80, 1-90, 1-95, 1-96, 1-97, 1-98, or 1-99 of SEQ ID NO: 16. In a particular embodiment, the light chain is a kappa chain. In another embodiment, a nucleic acid contains a sequence of nucleotides that encodes the sequence of amino acids 1-100 of SEQ ID NO: 16 and a J region. The J region can be from any species, including but not limited to mammals, such as, for example, primates, rodents and humans. For example,
15 the J can contain the sequence of amino acids set forth in SEQ ID NO: 46, 48, 50, 52, 54, 55, 57, 59, 61, 73, 75, 77 or 79. In a particular embodiment, the J region contains a sequence of amino acids from 101 to 112 as set forth in SEQ ID NO: 16. In another embodiment, a nucleic acid contains a sequence of nucleotides that encodes the sequence of amino acids 1-100 of SEQ ID NO: 16 and a constant (C) region. The C region can be
20 from any species, including but not limited to mammals, such as, for example, primates, rodents and humans. In particular embodiments, the C region is a light chain C region. For example, the C region can be a kappa light chain constant sequence. In one example, the C region can contain the sequence of amino acids set forth in SEQ ID NO: 63, 65 or 81. In another embodiment, a nucleic acid can further contain, in addition to a sequence
25 of nucleotides encoding a C region, a sequence of nucleotides encoding a variable region such as those described herein above. In a particular embodiment, a nucleic acid provided herein contains the sequence of nucleotides set forth in SEQ ID NO: 15 or the sequence of nucleotides 1-300 set forth in SEQ ID NO: 15.

 In another embodiment, a nucleic acid contains a sequence of nucleotides that
30 encodes the amino acid sequence set forth in SEQ ID NO: 18. In a particular

- 137 -

embodiment, a nucleic acid contains a sequence of nucleotides that encodes the amino acid sequence set forth as amino acids 1-114 or 1-98 of SEQ ID NO: 18. In one embodiment, a nucleic acid contains a sequence of nucleotides that encodes a heavy chain variable region containing the sequence of amino acids 1-98 of SEQ ID NO: 18. In

5 a further embodiment, a nucleic acid contains a sequence of nucleotides that encodes a heavy chain variable region containing the sequence of amino acids selected from 1-50, 1-60, 1-70, 1-80, 1-90, 1-91, 1-92, 1-93, 1-94, 1-95, 1-96, or 1-97 of SEQ ID NO: 18. In a particular embodiment, the heavy chain is an IgG_{2a} heavy chain. In another embodiment, a nucleic acid contains a sequence of nucleotides that encodes the sequence

10 of amino acids 1-98 of SEQ ID NO: 18 and a DJ region. The DJ region can be from any species, including but not limited to mammals, such as, for example, primates, rodents and humans. For example, the DJ can contain the sequence of amino acids set forth in SEQ ID NO: 67, 89 or 91. In a particular embodiment, the DJ region contains a sequence of amino acids from 99 to 114 as set forth in SEQ ID NO: 18. In another

15 embodiment, a nucleic acid contains a sequence of nucleotides that encodes the sequence of amino acids 1-98 of SEQ ID NO: 18 and one or more a constant (C) regions. The C region can be from any species, including but not limited to mammals, such as, for example, primates, rodents and humans. In particular embodiments, the C region is a heavy chain C region. For example, the C region can be a heavy chain C_γ region. For

20 example, the C region can contain the sequence of amino acids set forth in SEQ ID NO: 69, 71, 83, 85 or 87. In a particular example, the C region can be an IgG_{2a} heavy chain constant sequence. In another embodiment, a nucleic acid can further contain, in addition to a sequence of nucleotides encoding a C region, a sequence of nucleotides encoding a variable region such as those described herein above. In a particular

25 embodiment, a nucleic acid provided herein contains the sequence of nucleotides set forth in SEQ ID NO: 17 or the sequence of nucleotides 1-294 set forth in SEQ ID NO: 17.

Nucleic acid constructs, including, for example, plasmids and expression vectors, are also provided herein. In one embodiment of a nucleic acid construct provided herein, the nucleic acid contains a sequence of nucleotides encoding the sequence of amino acids

30 set forth in SEQ ID NO: 16 (or amino acids 1-112 or 1-100 of SEQ ID NO: 16) and the

sequence of amino acids set forth in SEQ ID NO: 18 (or amino acids 1-114 or 1-98 of SEQ ID NO: 18). In a another embodiment, a nucleic acid provided herein contains the sequence of nucleotides set forth in SEQ ID NO: 15 and SEQ ID NO: 17. In another embodiment, a nucleic acid provided herein contains the sequence of nucleotides set forth in SEQ ID NO: 15 and SEQ ID NO: 17 or a sequence of nucleotides encoding the sequence of amino acids set forth in SEQ ID NO: 16 (or amino acids 1-112 or 1-100 of SEQ ID NO: 16) and a sequence of nucleotides encoding the sequence of amino acids set forth in SEQ ID NO: 18 (or amino acids 1-114 or 1-98 of SEQ ID NO: 18) and further contains one or more sequences of nucleotides encoding one or more of the following amino acid sequences: a J region, *e.g.*, a light or a heavy chain J region, including, for example, a kappa light chain J region and a γ heavy chain J region, and a C region, *e.g.*, a light chain or heavy chain constant region, including, for example, a kappa light chain constant region, and a γ heavy chain C region, such as an IgG_{2a} heavy chain constant region. These regions can be from any species, including, but not limited to, mammals, such as, for example, primates, rodents and humans. Exemplary amino acid sequences of such regions can be any of those described herein above or known in the art.

Antibodies that bind substantially similarly to any A β peptide which contains an amino-terminal sequence substantially as set forth in the sequence of amino acids 1-12 of SEQ ID NO: 4 can be generated using animal immunization or recombinant DNA protocols described herein or known in the art. For example, such an antibody provided herein, referred to as B436 (see EXAMPLES 2 and 4), was generated by designing a peptide immunogen having the sequence DAEFRHDSGYEV-C that represents the A β 1-12 region. The resulting murine monoclonal antibody was determined to have high titer for both A β 40 and A β 42 peptides.

25 **iii. A β 40 antibody**

Antibodies that bind A β 40 (e.g., a form of A β containing the sequence of amino acids 1-40 of SEQ ID NO: 4) were also generated for use in methods described herein. For example, such antibodies can be used in particular embodiments of the methods for identifying agents that modulate A β 42 levels. In these embodiments, which are described herein, a sample containing APP and/or a portion(s) thereof is contacted with a

- 139 -

test agent and an agent is identified that selectively modulates A β 42 levels relative to A β 40 levels. In a particular method, the A β 42 levels of a sample are assessed using an A β 42-selective antibody, such as provided and described herein, and the A β 40 levels are assessed using an antibody that binds A β 40.

5 An A β 40 antibody was produced using animal immunization protocols as described herein. The A β 40 antibody was prepared using the same protocol as described herein for production of antibody A387 (an A β 42-selective antibody) production except that the peptide C-AIIGLMVGGVV (the sequence of amino acids 30-40 of SEQ ID NO: 4) was used to conjugate to ovalbumin and immunize mice. Subsequent titering was
10 performed as described for the A β 42-selective antibody.

(b) A β Assays

Immunoassays for detecting protein are well known to those of skill in the art. Exemplary immunoassay formats include ELISA, competitive immunoassays, radioimmunoassays, Western blots, indirect immunofluorescent assays, *in vivo*
15 expression or immunization protocols with purified protein preparations. In general, an immunoassay to detect a protein or peptide involves contacting a cell-based or cell-free sample with the antibody of interest and incubating for a period of time sufficient to allow binding of antibody to the epitope, usually at least about 10 minutes. Detection of immunocomplex formation is well known in the art and may be achieved by methods
20 generally based upon the detection of a label or marker, such as any of the radioactive, fluorescent, luminous, biological or enzymatic tags. Examples of the radioisotopes include ¹²⁵I, ¹³¹I, ³H and ¹⁴C. Enzymatic tags that are stable and have a high specific activity are particularly suited for these methods. Examples of enzymatic tags include β -galactosidase, β -glucosidase, alkaline phosphatase, peroxidase, and malate
25 dehydrogenase. Examples of fluorescent tags include fluorescamine and fluorescein isothiocyanate. Luminous tags include, for example, luminol, luminol derivatives, luciferin and lucigenin. Labels are well known to those skilled in the art (see U.S. Pat. Nos. 3,817,837; 3,850,752; 3,939,350; 3,996,345; 4,277,437; 4,275,149 and 4,366,241, each incorporated herein by reference).

30 A primary antibody may be directly labeled with radioisotopes, enzymes,

- 140 -

fluorescers, chemilumescers, or other labels for direct detection. Alternatively, a secondary binding ligand such as a second antibody or a biotin/avidin ligand-binding arrangement may be used. The secondary ligand or reagent may be useful for amplifying the signal. Such reagents are well known in the art. For example, the primary antibody
5 may be conjugated to biotin, with horseradish peroxidase-conjugated avidin added as a second stage reagent. Final detection uses a substrate that undergoes a color change in the presence of the peroxidase. The absence or presence of antibody binding may be determined by various methods, including flow cytometry of dissociated cells, microscopy, radiography, scintillation counting, luminometer, etc.

10 Detection and measurement of A β peptides can involve the use of a two-site or "sandwich" assay employing two antibodies, one antibody capable of distinguishing an A β peptide (*e.g.*, A β 42) from other A β peptides that might be found in the sample and a second antibody. One of the antibodies serves to capture the antigen while the other is used to detect the captured antigen or the antibody-antigen complex. Thus, for example,
15 an antibody that is selective for a particular A β (*e.g.*, A β 42) can be used as a capture antibody while an antibody that binds A β peptides either non-selectively or selectively, is used as a detection antibody. The first and second antibody reactions may be conducted simultaneously or sequentially. The detection antibody is conjugated to a detectable label as described above. In a particular embodiment, the detectable label is an
20 enzymatic tag. In a further embodiment, the label is alkaline phosphatase and the presence or absence of antibody binding is determined by luminescence of a substrate that undergoes a color change in the presence of alkaline phosphatase, such as, for example, CDP-Star chemiluminescence substrate (Tropix, Inc.). Further, in the sandwich immunoassay methods, the A β selective antibodies or the antibodies used for labeling are
25 not necessarily of one kind, but two or more kinds of antibodies may be used as mixtures for the purpose of enhancing the measuring sensitivity.

In an example of a method for detecting or measuring A β by the sandwich technique, the anti-A β antibody used in the first reaction can be reactive to a portion(s) of the A β peptide that is different from the portion(s) that the antibody used in the second
30 reaction recognizes. For example, when the antibody used in the first reaction recognizes

- 141 -

a partial peptide on the C-terminal portion of A β , the antibody used in the second reaction generally is one that recognizes a partial peptide other than the partial peptide on the C-terminal portion (for example, a partial peptide on the N-terminal portion of A β).

In a particular embodiment of a method for detecting A β 42 and/or measuring
5 A β 42 levels or determining the A β 42 content of a sample provided herein, an A β 42-selective antibody or portion or fragment thereof provided herein is used as the antibody of the first reaction in the sandwich assay (primary antibody). For example, the A β 42-selective antibody, or portion or fragment thereof, can be one that contains the sequence of amino acids 1-95 of SEQ ID NO: 12 and/or the sequence of amino acids 1-97 of SEQ
10 ID NO: 14. The secondary antibody can be any antibody that recognizes an epitope within the A β 42 peptide. In one embodiment, the secondary antibody reacts with a portion(s) of A β 42 that is different than the site(s) at which the primary antibody reacts. In a particular embodiment, the antibody of the second reaction in the sandwich assay (secondary antibody) is reactive with an N-terminal portion of A β 42. The secondary
15 antibody (or portion or fragment thereof) can be one that is reactive to more than one species of A β and can be reactive with most if not all forms of A β . In a particular embodiment, the secondary antibody (or portion or fragment thereof) is reactive with A β peptides containing amino acids 1-12 of SEQ ID NO: 4. For example, the secondary antibody, or portion or fragment thereof, can be one that contains the sequence of amino
20 acids 1-100 of SEQ ID NO: 16 and/or the sequence of amino acids 1-98 of SEQ ID NO: 18. The secondary antibody can be used as the detection antibody and can be conjugated to a detectable label. In a particular embodiment, the detectable label is alkaline phosphatase and the presence or absence of antibody binding is determined by luminescence of a substrate that undergoes a color change in the presence of alkaline
25 phosphatase, such as, for example, CDP-Star chemiluminescence substrate (Tropix, Inc.).

When a method for detecting A β 42 and/or measuring A β 42 levels or determining the A β 42 content of a sample as provided herein is used as method for assessing the A β 42 levels in a step of identifying an agent that selectively modulates A β 42, it may be combined with a method for detecting and/or measuring A β 40 in a sample, as described
30 herein. In such methods for identifying agents that selectively modulate A β 42 levels, the

- 142 -

A β 42 level of one or more samples is assessed to identify an agent that modulate A β 42 levels, and the A β 40 level of one or more samples is assessed to identify those A β 42-modulating agents that do not alter A β 40 levels. One method for detecting and/or measuring A β 40 in a sample for use in these methods is the above-described sandwich assay wherein an A β 40-selective antibody (or portion or fragment thereof) is substituted for an A β 42-selective antibody or portion or fragment thereof as the primary antibody. In a particular embodiment, the A β 40-selective antibody is one that recognizes amino acids 30-40 of A β (for example, amino acids 30-40 of SEQ ID NO: 4), such as is described herein. For example, an A β 40-selective antibody can be prepared by immunizing animals with the peptidyl sequence representing A β 30-40 region, as described herein.

Sandwich ELISA-based assays such as these for use in methods for detecting A β 42 and/or measuring A β 42 levels or determining the A β 42 content of a sample as provided herein can be performed in a microtiter plate format wherein the primary antibody is coated into the wells of the plate and the sample is added to the wells. After washing, the secondary antibody (which can be conjugated to a label such as alkaline phosphatase) is added to the wells which are washed prior to adding a substrate, *e.g.*, a chemiluminescent substrate, for detection of bound A β 42. Such methods provide a large linear range, such as, for example, about 75-2000 pg/well, high dynamic range, *e.g.*, about 3-30 fold over background in linear range (signal:noise), low sensitivity limit, such as, for example, less than about 20 pg/well, and selectivity for A β 42, *e.g.*, at least about 1000-fold selectivity for A β 42 over other A β peptides, making the method highly amenable to high-throughput screening for agents that modulate A β 42 levels.

Smaller A β peptides, for example, A β peptides having a C-terminal end that terminates before amino acid 40 (see, *e.g.*, the sequence of amino acids 1-40 of SEQ ID NO: 4) may also be detected in the methods provided herein. In particular embodiments, these peptides are measured by their mass, size, and/or charge. For example, peptides may be immunoprecipitated with an antibody reactive to the amino-terminal end of A β . For example, the anti-A β 1-12 antibody described herein may be used for immunoprecipitation of these peptides. Immunoprecipitated peptides may then be identified by any method known to those of skill in the art including, for example,

- 143 -

electrophoresis and mass spectrometry. In a particular embodiment, cells expressing wild-type APP are treated with test agent or vehicle control for 18 h. Media is collected and immunoprecipitated using an anti-A β 1-12:Sepharose[®] column for 4 h. Bound peptides are eluted with 0.1% TFA/50% acetonitrile and spotted onto NP2 CHIPS. Mass spectrometer analysis is performed on a PBS II Protein Chip Reader (Ciphergen). Data may be normalized to an internal standard, such as A β ₁₋₁₁ that is spiked into the media prior to the immunoprecipitation.

All assays and procedures, including antibody-antigen reactions, generally can be conducted under conditions recognized by those of skill in the art as standard conditions.

10 **d. Alterations of APP cleavage or processing, A β processing or A β levels**

Methods for identifying or screening for agents that modulate A β levels can include a step of identifying an agent that alters cleavage (particularly the A β peptide-producing cleavage) of APP (and/or portion(s) thereof), processing of APP (and/or portion(s) thereof), A β processing and/or A β levels of a sample. The step of identifying an agent that alters such parameters that can affect A β levels typically involves making assessments of one or more of the parameters. As described herein, there are a number of ways in which APP cleavage, APP processing, A β processing and the A β levels of a sample can be assessed, including, but not limited to, immunoassays for detection and/or quantitation of one or more peptides, proteins and/or fragments thereof that are reflective of these parameters. A step of identifying an agent that alters one or more of these parameters can thus involve assessment of one or more of the parameters and a determination as to whether the parameter(s) is altered under a condition of the presence of the agent.

25 Determining if APP cleavage (particularly the A β peptide-producing cleavage), APP processing, A β processing and/or A β levels of a sample is altered by a test agent can involve comparing one or more of these parameters in the presence and absence of the test agent. Thus, in general, the agent identification step can involve a comparison of the cleavage (particularly the A β peptide-producing cleavage) of APP (and/or portion(s) thereof), processing of APP (and/or portion(s) thereof), A β processing and/or A β levels

30

- 144 -

of a sample that has been contacted with a test agent (i.e., test sample) and of a sample that has not been contacted with the test agent (i.e., control sample). If the A β -producing cleavage or processing of APP (and/or portion(s) thereof), the processing of A β and/or the A β levels of the test and control samples differ, then the agent is identified as one that modulates the level of one or more A β peptides.

(1) Contacting sample with test agent

A sample for use in the methods of identifying or screening for agents that modulate A β levels as described herein can be maintained under conditions in which APP (and/or portion(s) thereof, including A β) can undergo cleavage and/or processing (e.g., catabolism, degradation). If the sample is a test sample, it is contacted with a test agent. If the sample is a control sample, it can be one that is not contacted with test agent. Generally, a control sample is substantially similar to the test sample and maintained under substantially similar conditions as the test sample, but is not contacted with test agent. A control sample can be the same physical sample as the test sample (e.g., prior to addition of test agent) or can be a different sample.

Depending on the type of control (e.g., reference control, negative control or positive control), a control sample may be manipulated in various ways. For example, if a control sample is a vehicle control, it may be contacted with a "vehicle", such as a medium, or element thereof, in which the test agent is contained, but that lacks the test agent. Examples of such "vehicles" include suspension, solubilizing reagents, emulsifiers, and compositions that generally serve to facilitate retention and administration of a test agent. In a particular example, a vehicle control can be DMSO. A positive control can be a sample that has been treated using known processes/compositions to achieve an effect that is desired by a test agent that is a "positive" identified as one that modulates A β levels. Thus, for example, if the methods are conducted with the specific purpose of identifying an agent that reduces one or more A β levels, then a positive control sample could be one that is treated with an agent known to reduce A β levels. One particular example is an APP-containing sample that has been contacted with a β - and/or γ -secretase inhibitor, such as, for example, DAPT.

Test samples can be treated with a range of doses or concentrations of the test

- 145 -

agent or with only a single concentration of agent. When a range of different test agent concentrations is used in contacting a plurality of samples in parallel and compared to the magnitude of any effect each different concentration may have on the parameter(s) (*e.g.*, A β level of samples) being assessed (*e.g.*, a dose-response study), a more detailed analysis and profile of the test agent can be made. For example, it may be possible to determine values such as EC₅₀ or IC₅₀ for the test agent to estimate the potency of an agent. The methods provided herein allow for the identification of very potent A β -modulating agents. Particular embodiments of the methods provide for the identification of agents with an EC₅₀ or IC₅₀ for modulating (*e.g.*, increasing or decreasing) A β levels of 100 μ M, 75 μ M, 50 μ M, 40 μ M, 30 μ M, 25 μ M, 20 μ M, 15 μ M, or 10 μ M or lower. In a particular embodiment of the methods, agents are identified that have an EC₅₀ or IC₅₀ for modulating (*e.g.*, increasing or decreasing) A β levels of less than about 25 μ M. In a further embodiment of the methods, agents are identified having an EC₅₀ or IC₅₀ of less than about 20 μ M. In one particular embodiment, agents are identified that have such values for an IC₅₀ for reducing the levels of A β 42. Generally, such a more detailed analysis is conducted after a test agent has been identified as one that alters one or more of the parameters at a threshold or test concentration, such as is typically done in a high-throughput screening of test agents. Threshold or test concentrations can be, for example, about 1 μ M, 2 μ M, 5 μ M, 10 μ M, 15 μ M, 20 μ M, 25 μ M, 30 μ M, 35 μ M, 40 μ M, 45 μ M, 50 μ M, 75 μ M, 100 μ M or more. In a one example, the threshold or test concentration can be less than about 50 μ M, 40 μ M, 35 μ M, 30 μ M, 25 μ M, 20 μ M, 15 μ M or 10 μ M. In a particular example, the threshold or test concentration can be less than or equal to about 35 μ M, 30 μ M, 25 μ M, 20 μ M, 15 μ M or 10 μ M. Generally, by screening at a lower test concentration, the agents identified as modulators of A β may tend to be more potent than if they had been identified at a higher test concentration.

A sample for use in the methods of identifying or screening for agents that modulate A β levels as described herein can be maintained under conditions in which APP (and/or portion(s) thereof, including A β) can undergo cleavage and/or processing (*e.g.*, catabolism, degradation) for an appropriate amount of time prior to being used in the methods of identifying A β -modulating agents and after being contacted with test

- 146 -

agent. Such time periods can be empirically determined and generally are such to allow for detectable levels of APP cleavage or processing and/or A β formation or processing to occur. Similarly, the sample is contacted with test agent for an appropriate amount of time or range of time periods. Typically, when the methods are being practiced in a high-throughput screening format, a single time period of contacting is used. In one example, the time period can be on the order of minutes to hours depending, in part, on the type of sample, *e.g.*, intact cells or cell-free medium.

In one particular example of a method for identifying an agent that modulates A β levels, cell cultures capable of APP expression and processing (*e.g.*, CHO cells transfected with DNA encoding human APP695 and human PS1) are plated in the wells of a microtiter plate and allowed to adhere for about 24 hours. The separate samples in the wells were then either treated or not treated with a test agent (~ 30 μ M). Samples treated with DMSO vehicle (0.12%) alone were a negative control. Samples treated with 1 μ M DAPT for 18 hours were used as a positive control. Supernatant removed from the wells was analyzed in a sandwich ELISA to assess the level of A β 42 in each sample. The ELISA was conducted in a microtiter plate format using an A β 42-selective monoclonal antibody provided herein (antibody A387) as a capture antibody which was incubated with supernatant for 1 hour. After washing of the plate, the wells were incubated for 2 hours with a detection antibody generated against an A β 1-12 peptide, as described herein, and conjugated to alkaline phosphatase. A chemiluminescence substrate was added to the wells and, after 30 minutes, the luminescence was quantified to assess and compare A β 42 levels of the test and control samples in order to determine any differences and identify agents that modulate A β (and in particular A β 42) levels.

(2) Evaluating alterations

In conducting the methods of identifying an agent that modulates A β levels, a way in which an agent can be identified is by identifying an agent that alters the cleavage or processing of APP (and/or portion(s) thereof), the processing of A β and/or A β levels. An alteration can be, for example, any detectable difference in the cleavage or processing of APP (and/or portion(s) thereof), the processing of A β and/or A β levels of a sample that has been contacted with a test agent as compared to a sample that has not been

- 147 -

contacted with the test agent. Methods for assessing the cleavage or processing of APP (and/or portion(s) thereof), the processing of A β and A β levels are described and provided herein and additional assessment methods are known in the art. Thus, any difference in any one or more of these processes or compositions as detected in the
5 assessment of test and control samples can be an alteration by which an A β -modulating agent can be identified.

The extent of the difference can vary depending on a variety of factors, including, for example, the particular parameter being assessed and compared, the assessment method used and the conditions under which the assessment was conducted, the
10 concentration of the test agent used as well as other factors. Thus, for example, the difference may be an about 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75 or more than 75% difference in the assessed parameter, *e.g.*, a level or amount of composition, activity or processing, when compared under differing conditions, *e.g.*, in the presence and absence of a test agent. In one example of a particular high-throughput format of the
15 methods, a test agent was identified as an agent that modulates A β 42 levels if there was a greater than about 50% difference in the A β 42 levels of test and control samples. In a particular example, a test agent was identified as an agent that reduces A β 42 levels if the A β 42 level of a test sample was more than about 50% lower than the A β 42 level of a control sample.

20 **e. Assessment of selectivity of A β -modulating agents**

Cellular and extracellular A β levels are governed by numerous mechanisms and activities involved in A β synthesis through APP processing and in A β catabolism, degradation and clearance. These mechanisms include multiple components, such as, for example, enzymes and facilitator proteins, many of which have multiple substrates
25 and/or multiple, closely related protein family members. In addition, some of the enzymes, *e.g.*, γ -secretase, may function as a part of a complex that includes a number of other proteases. Although any of these components and mechanisms, individually or in combination, are potential targets for modulation in order to ultimately modulate A β levels, modulation of these targets may also affect other processes (*i.e.*, other than the
30 processing of APP and/or A β) and the levels of other molecules due to the multiplicity of

- 148 -

component function and relatedness and interaction of some components to non-component molecules. Modulation of A β levels that also involves modulation of other cellular processes and elements, i.e., non-specific modulation of A β levels, can result in undesired side effects. Methods of identifying agents that more specifically or selectively
5 modulate A β levels are provided herein. The methods can be used to identify agents that selectively modulate the levels of one or more A β peptides without substantially affecting compositions and mechanisms that are not significantly involved in the generation, degradation and/or clearance of one or more A β peptides.

Using the methods provided herein, agents identified as A β -modulating agents
10 can also be profiled with respect to the specificity or selectivity of their modulation.

(1) Assessment of A β peptide selectivity

Cleavage of APP to generate A β yields a number of A β peptides that can differ at the C-terminus, *e.g.*, A β 1-43, A β 1-42, A β 1-40, and others. The C-terminal heterogeneity is the result of cleavage by distinct activities of γ -secretase and/or multiple γ -secretases.
15 An agent that modulates the levels of all or most or more than one or two A β peptides may be non-selectively modulating components and mechanisms involved in processes other than the generation or degradation of A β in addition to modulating components and mechanisms of A β synthesis and degradation. Agents that selectively modulate the levels of one or two A β peptides, or a particular subset of A β peptides, are less likely to
20 affect other compositions, activities and mechanisms and are therefore desired. Agents that selectively modulate the level of A β 42 are of particular interest because A β 42 is one of the predominant forms found in amyloid plaques, and is deposited early and selectively in the cerebral cortex of brains of individuals harboring some FAD-linked mutations. A β 42 formation is also selectively elevated in some FAD-linked mutations.

25 Methods are provided herein for identifying or screening for an agent that selectively modulates A β levels. In one embodiment, the method identifies agents that alter the level of a particular form or forms of A β to a greater extent than they alter the levels of one or more other forms of A β . In a particular embodiment, such agents alter the level of a particular form or forms of A β without substantially affecting or altering
30 the level of one or more other A β peptides. In one embodiment of the methods, an agent

- 149 -

that selectively modulates A β 42 levels is identified. The agent can, for instance, selectively modulate A β 42 levels relative to A β 40 levels and/or the levels of all or most of the other forms of A β . In a particular embodiment, the agent modulates the levels of A β 42 and A β 39 relative to A β 40 levels and/or the levels of all or most of the other forms of A β . In a particular embodiment of the method, compounds are identified that selectively modulate A β peptides having a C-terminal end that terminates before amino acid 40. In a particular embodiment compounds are identified that selectively modulate the level of A β 39. In a particular embodiment, the methods identify an agent that selectively increases A β 39 levels. In another embodiment the methods identify an agent that selectively decreases A β 39 levels.

In general, the methods of identifying or screening for an agent that selectively modulates the level of an A β peptide relative to one or more other A β peptides includes steps of contacting a sample containing APP and/or a portion(s) thereof (including, for example one or more A β peptides) with a test agent and identifying an agent that alters the level of an A β peptide to a greater extent than it alters the level of one or more other A β peptides. The process of identifying an agent that modulates the level of an A β peptide in a sample can be carried out in a number of ways as described herein. For example, the A β peptide level in a sample that has been contacted with a test agent (test sample) can be compared with the A β peptide level in a sample that has not been contacted with the test agent (control sample). If the A β peptide levels in the two samples differ, then the agent is identified as one that modulates the level of the A β peptide. Methods for assessing the level of a particular A β peptide in a sample are described herein or known in the art. Such methods include, but are not limited to, immunoassays employing peptide-specific antibodies, mass spectrometry and electrophoretic analyses.

In a particular embodiment of the methods for identifying or screening for an agent that selectively modulates the level of an A β peptide, the A β 42 levels of samples are assessed by contacting a sample with an antibody (or portion or fragment thereof) that selectively binds to A β 42 (*e.g.*, the sequence of amino acids 1-42 of SEQ ID NO: 4). In one embodiment, the antibody is any one of the A β 42-selective antibodies provided

- 150 -

herein, such as, for example, an antibody that contains the sequence of amino acids 1 to about 95, 94, 93, 92, 91, 90, 85, 80, 70, 60 or 50 of SEQ ID NO: 12 and/or the sequence of amino acids 1 to about 97, 96, 95, 94, 93, 92, 91, 90, 85, 80, 70, 60 or 50 of SEQ ID NO: 14. In a particular embodiment, the A β 42-selective antibody used in assessing the

5 A β 42 levels of samples is antibody A387 provided herein. In another embodiment of the methods for identifying or screening for an agent that selectively modulates the level of an A β peptide, the A β 39 levels of samples are assessed by contacting a sample with an antibody (or portion or fragment thereof) that selectively binds to A β 39 (e.g., the sequence of amino acids 1-39 of SEQ ID NO: 4) or by mass spectrometric analysis of the

10 samples. Antibodies selective for A β 39 can be prepared using methods described herein. In particular methods, an agent that modulates A β 42 levels or A β 39 levels or that modulates both A β 42 and A β 39 levels is identified. In a particular method, an agent that reduces A β 42 levels and/or increases A β 39 levels is identified.

The process of further identifying an agent that alters the level of one or more

15 other A β peptides to a lesser extent than it alters a particular A β peptide or that does not substantially alter the level of the one or more other A β peptides can also be carried out in a number of ways. In general, this process can involve a comparison of the level of one or more other A β peptides in a sample that has been contacted with the agent (test sample) with that of a sample that has not been contacted with the agent (control

20 sample). If the difference in the levels of the one or more other A β peptides in the test and control samples is less than the difference in the levels of the particular modulated A β peptide in test and control samples, or if the levels of the one or more other A β peptides in the test and control samples do not differ substantially (or are substantially unchanged), then the agent is identified as one that selectively modulates the level of an

25 A β peptide. The skilled artisan can select appropriate concentrations of test agents at which to make such comparisons. For example, the comparison can be made at or near the EC₅₀ or IC₅₀ concentration for the modulation of the target A β peptide. If the method is for identifying an agent that selectively modulates the level of an A β peptide relative to only one other A β peptide, then the process of assessing the extent to which an agent may

30 alter the levels of the one other A β peptide can involve an assessment of the levels of the

- 151 -

one other peptide in test and control samples using an antibody selective for the one other peptide. If the method is for identifying an agent that selectively modulates the level of an A β peptide relative to most or all other A β peptides, then the process of assessing the extent to which an agent can alter the levels of most or all other A β peptides can involve
5 an assessment of the levels of all A β peptides in test and control samples using an antibody that recognizes most or all forms of A β . If the ratio of the level of the modulated A β peptide to the level of all A β peptides differs in the control and test samples, then the agent is identified as one that selectively modulates the level of the modulated A β peptide relative to most or all other A β peptides. In a particular
10 embodiment of such a method, the antibody that recognizes most or all forms of A β in a sample is one that binds to A β 1-12 (*e.g.*, the sequence of amino acids 1-12 of SEQ ID NO: 4). In one embodiment, the antibody is one that is provided herein, such as an antibody that contains the sequence of amino acids 1 to about 100, 99, 98, 97, 96, 95, 94, 93, 92, 91, 90, 85, 80, 70, 60 or 50 of SEQ ID NO: 16 and/or the sequence of amino
15 acids 1 to about 98, 97, 96, 95, 94, 93, 92, 91, 90, 85, 80, 70, 60 or 50 of SEQ ID NO: 18. In a particular embodiment, the A β 1-12 antibody used in assessing the A β peptide levels of samples is antibody B436 provided herein.

In method of identifying an agent that selectively modulates the level of an A β peptide relative to one or more other A β peptides, the identification of the A β -
20 modulating agent and the determination as to what extent, if any, the agent alters the level of one or more other A β peptides can be conducted sequentially or simultaneously. For example, an agent that modulates the levels of an A β peptide can be identified by a difference in the levels of the A β peptide in samples contacted with the agent (test sample) and samples not contacted with the agent (control samples). That agent can then
25 be separately evaluated for its effects on the levels of one or more other A β peptides by comparing the levels of the one or more other A β peptides in samples contacted with the agent and not contacted with the agent. Alternatively, the levels of the particular A β peptide to be modulated and the levels of the one or more other A β peptides in a test sample can be assessed and compared to the levels of the particular A β peptide to be
30 modulated and the levels of the one or more other A β peptides in a control sample

- 152 -

simultaneously to, in one step, identify an agent that selectively modulates the level of an A β peptide.

In another method for identifying an agent that selectively modulates the level of an A β peptide relative to one or more other A β peptides, the test agent is one that is
5 already known to modulate the level of one or more particular A β peptides. Thus, in one embodiment of this method, a sample containing APP or portion(s) thereof is contacted with a test agent that modulates the level of an A β peptide, and a test agent is identified as an agent that selectively modulates A β levels if the test agent does not substantially alter the level of one or more A β peptides other than the A β peptide that is modulated by
10 the test agent. As described herein, the agent that modulates the level of an A β peptide that is used in this method can be one that was identified by a process involving contacting a sample containing APP or portion(s) thereof with a test agent and identifying an agent that alters the cleavage of APP that produces one or more A β peptides, the processing of APP and/or the level of one or more A β peptides.

15 An agent that selectively modulates the levels of an A β peptide relative to one or more other A β peptides can alter the levels of the selectively modulated A β peptide(s) to a greater extent than it alters the levels of the one or more other A β peptides (i.e., the peptides that are not targeted for modulation). The extent to which the agent alters the levels of the particular A β peptide is generally significantly greater than the extent to
20 which the agent alters the levels of one or more other A β peptides; that is, the greater extent of modulation is reproducible and not merely within the level of experimental error or variation. The modulation of a particular A β peptide by the agent can be identified by a detectable difference in the levels of the A β peptide in samples contacted with the agent (test sample) and samples not contacted with the agent (control samples).
25 The agent is one that selectively modulates the levels of the particular A β peptide if any difference (including, for example, absolute and/or percentage difference) in the levels of one or more other A β peptides in samples contacted with the agent and samples not contacted with the agent is less than the difference (including, for example, absolute and/or percentage difference) in the levels of the particular A β peptide in test and control
30 samples. In particular embodiments, the extent to which the agent alters the levels of one

- 153 -

or more other A β peptides (i.e., the peptides that are not targeted for modulation) is less than about 40%, 35%, 30%, 25%, or 20%. In one embodiment, the extent to which the agent alters the levels of one or more other A β peptides (i.e., the peptides that are not targeted for modulation) is less than 20%.

5 In a particular embodiment of the methods of identifying an agent that selectively modulates the level of an A β peptide, an agent is identified that modulates the level of an A β peptide without substantially altering the levels of one or more other A β peptides. Any modulation of the level of the one or more other A β peptides (i.e., the peptides that are not targeted for modulation) that is not a substantial alteration is one that is generally
10 not associated with any significant undesired or adverse consequence in a biological context, such as, for example, in a cell, cell medium, tissue or organism.

(2) **Assessment of presenilin substrate selectivity**

 An agent that modulates A β levels may act by modulating any one or more of the numerous mechanisms and activities, and components thereof, involved in A β synthesis
15 through APP processing and in A β catabolism, degradation and clearance. One activity involved in the generation of A β is the presenilin/ γ -secretase that participates in the processing and cleavage of APP. Any non-specific modulation of this activity could possibly effect other mechanisms in addition to APP cleavage due to the multiplicity of substrates and mechanisms with which presenilin and γ -secretase are involved. Such
20 non-specific actions of an A β -modulating agent could result in undesired and adverse side effects of the modulation process.

 Agents that more specifically or selectively modulate A β levels can be identified using methods provided herein that involve identifying agents that modulate A β levels without substantially altering or affecting non-APP substrate cleaving/processing activity
25 of presenilin. These methods can involve the methods of assessing presenilin and/or presenilin-dependent activity provided and described herein.

 One method provided herein for identifying or screening for agents that selectively modulate A β levels includes steps of contacting a sample containing a presenilin substrate, and/or portion(s) thereof, other than APP with a test agent that
30 modulates A β levels and identifying a test agent as an agent that selectively modulates

- 154 -

A β levels if the agent does not substantially alter the cleavage and/or processing (in particular, the presenilin-dependent cleavage and/or processing) of the presenilin substrate, and/or portion(s) thereof, that is other than APP. The sample used in this method can contain presenilin. The agent that modulates A β levels that is used in this

5 method can be any agent known to modulate A β levels. The agent can, for example, be one that is identified by a method described herein which involves contacting a sample containing APP and/or portion(s) thereof with a test agent and identifying an agent that alters the cleavage of APP that produces one or more A β peptides, the processing of APP and/or the level of one or more A β peptides.

10 The step of identifying an agent that does not substantially alter the cleavage and/or processing of the presenilin substrate, or portion(s) thereof, that is other than APP can be carried out in a number of ways. In general, this process can involve a comparison of the cleavage and/or processing (in particular, the presenilin-dependent cleavage and/or processing) of a presenilin substrate (and/or portion(s) thereof) other

15 than APP, and/or the levels of a peptide fragment(s) of the presenilin substrate, in a sample that has been contacted with the test agent (i.e., test sample) and in a sample that has not been contacted with the test agent (i.e., control sample). If the cleavage and/or processing of the presenilin substrate that is other than APP, and/or the substrate fragment(s) levels, of the test and control samples do not differ substantially, then the

20 agent is identified as one that alters the level of one or more A β peptides without substantially altering the cleavage of the presenilin substrate, or portion(s) thereof, that is other than APP. The control sample can be the same physical sample as the test sample or a different sample. When the control and test samples are the same, the control is the sample in the absence of test agent.

25 In a particular embodiment, the cleavage and/or processing of the presenilin substrate that is other than APP, and/or the substrate fragment(s) levels, of the test sample can be compared to that of a positive control sample. A positive control sample can be a sample that has been contacted with a known modulator of presenilin or presenilin-dependent activity. In one example, the known modulator is an inhibitor of

30 presenilin or presenilin-dependent activity. A particular example is DAPT, which is an

- 155 -

inhibitor of presenilin-dependent γ -secretase activity. When a positive control is used, an agent is identified as one that alters the level of one or more $A\beta$ peptides without substantially altering the cleavage of the presenilin substrate, if the cleavage and/or processing of the presenilin substrate and/or the substrate fragment(s) levels of the test sample differ significantly and/or substantially from that of the positive control sample. For example, a substrate fragment(s) level of the test sample can differ from that of the positive control such that the test sample levels are less than about 40%, 35%, 30%, or 20% of the positive control sample levels. In a particular embodiment the test sample levels can be less than or equal to about 20% of the control sample levels. In one such embodiment, the positive control sample is one that has been contacted with DAPT (with a presenilin substrate fragment level set as 100%) and the test sample levels of the fragment are less than or equal to about 20% of the positive control sample levels.

In a particular embodiment, the agent that modulates $A\beta$ levels that is used in the method is one that modulates the levels of $A\beta_{42}$. In a further embodiment, the agent can be one that selectively modulates the levels of $A\beta_{42}$ relative to $A\beta_{40}$ levels and/or the levels of all or most of the other forms of $A\beta$. In a particular embodiment, the agent modulates the levels of $A\beta_{42}$ and $A\beta_{39}$ relative to $A\beta_{40}$ levels and/or the levels of all or most of the other forms of $A\beta$. In one embodiment, the agent reduces $A\beta_{42}$ levels and/or increases $A\beta_{39}$ levels. Thus, using the agent identification and screening methods provided herein in combination, it is possible to identify agents that reduce $A\beta_{42}$ levels without substantially altering the levels of $A\beta_{40}$ or the non-APP substrate cleavage/processing activity of presenilin (i.e., with an inhibitory profile ($A\beta_{42}(+)$, $A\beta_{40}(-)$, presenilin $(-)$)).

Another method provided herein for identifying or screening for an agent that selectively modulates $A\beta$ levels includes steps of contacting a sample containing APP and/or a portion(s) thereof (including, for example one or more $A\beta$ peptides) and a presenilin substrate, and/or portion(s) thereof, that is other than APP with a test agent and identifying an agent that alters the $A\beta$ peptide-producing cleavage or processing of the APP (and/or portion(s) thereof), the processing of $A\beta$ and/or the levels of one or more $A\beta$ peptides without substantially altering the cleavage (in particular, the presenilin-

- 156 -

dependent cleavage) of the presenilin substrate, or portion thereof, that is not APP. The sample used in this method can contain presenilin. The process of identifying an agent that alters the cleavage of APP that produces one or more A β peptides, the processing of APP or A β , and/or the level of one or more A β peptides can be carried out in a number of ways as described herein.

The process of further identifying an agent that does not substantially alter the cleavage of a presenilin substrate (other than APP), or portion(s) thereof, can also be carried out in a number of ways, as described herein. In general, this process can involve a comparison of the cleavage and/or processing (in particular, the presenilin-dependent cleavage and/or processing) of a presenilin substrate (or portion(s) thereof) other than APP, and/or the levels of a peptide fragment(s) of the presenilin substrate, in a sample that has been contacted with the test agent (i.e., test sample) and in a sample that has not been contacted with the test agent (i.e., control sample). If the cleavage and/or processing of the presenilin substrate that is other than APP and/or the substrate fragment(s) levels of the test and control samples do not differ substantially, then the agent is identified as one that alters the level of one or more A β peptides without substantially altering the cleavage and/or processing of the presenilin substrate, or portion(s) thereof, that is other than APP. The control sample can be the same physical sample as the test sample or a different sample. When the control and test samples are the same, the control is the sample in the absence of test agent.

In a particular embodiment of these methods, the cleavage and/or processing of the presenilin substrate that is other than APP, and/or the substrate fragment(s) levels, of the test sample can be compared to that of a positive control sample. A positive control sample can be a sample that has been contacted with a known modulator of presenilin or presenilin-dependent activity. In one example, the known modulator is an inhibitor of presenilin or presenilin-dependent activity. A particular example is DAPT, which is an inhibitor of presenilin-dependent γ -secretase activity. When a positive control is used, an agent is identified as one that alters the level of one or more A β peptides without substantially altering the cleavage of the presenilin substrate, if the cleavage and/or processing of the presenilin substrate and/or the substrate fragment(s) levels of the test

- 157 -

sample differ significantly and/or substantially from that of the positive control sample. For example, a substrate fragment(s) level of the test sample can differ from that of the positive control such that the test sample levels are less than about 40%, 35%, 30%, or 20% of the positive control sample levels. In a particular embodiment the test sample
5 levels can be less than or equal to about 20% of the control sample levels. In one such embodiment, the positive control sample is one that has been contacted with DAPT (with a presenilin substrate fragment level set as 100%) and the test sample levels of the fragment are less than or equal to about 20% of the positive control sample levels.

In one method of identifying an agent that selectively modulates $A\beta$ levels, the
10 identification of the $A\beta$ -modulating agent and the determination as to whether the agent alters the cleavage and/or processing of a presenilin substrate (other than APP), and/or portion(s) thereof, can be conducted sequentially or simultaneously. For example, when conducting the processes sequentially, an agent that modulates $A\beta$ levels can be identified by a difference in the $A\beta$ -producing cleavage of APP, the processing of APP or
15 $A\beta$, and/or the level of one or more $A\beta$ peptides in samples contacted with the agent (test sample) and samples not contacted with the agent (control sample). The identified agent can then be separately evaluated for its effect on presenilin substrate cleavage by comparing the cleavage and/or processing (in particular, presenilin-dependent cleavage and/or processing) of the presenilin substrate and/or the levels of a peptide fragment or
20 fragments of the presenilin substrate in samples contacted with the test agent and not contacted with the test agent. In this sequential method, the sample used in the identification of the $A\beta$ -modulating agent can be of the same or different type relative to the sample used in the determination as to whether the agent alters the cleavage of a presenilin substrate. If the same type of sample is used, it can contain APP (and/or
25 portion(s) thereof) and a presenilin substrate (and/or portion(s) thereof) other than APP. The sample can also contain presenilin. If different types of samples are used, the sample used in the identification of the $A\beta$ -modulating agent can contain APP and/or portion(s) thereof, and the sample used in the determination of alteration in the cleavage of the presenilin substrate can contain a presenilin substrate (and/or portion(s) thereof) other
30 than APP. The sample may also contain presenilin.

- 158 -

Alternatively, in a simultaneously performed method, a test sample containing APP (and/or portion(s) thereof) and a presenilin substrate (and/or portion(s) thereof) other than APP can be contacted with a test agent, and the A β -producing cleavage of APP, the processing of APP and/or A β , and/or the level of one or more A β peptides can be assessed for the test sample, as can presenilin substrate cleavage be assessed for the same test sample. The sample may also contain presenilin. The A β peptide-producing cleavage or processing of APP, processing of A β and/or levels of A β peptides in the test sample, as well as the presenilin substrate cleavage of the test sample, can be compared to that of a control sample in one step to identify an agent that modulates A β levels without substantially altering the cleavage of a presenilin substrate (or portion(s) thereof). In particular embodiments of any of the methods, a step in the method can be identifying an agent that modulates A β 42 levels without substantially altering the cleavage and/or processing of a presenilin substrate that is other than APP. The step can include identifying an agent that modulates A β 42 levels relative to A β 40 levels and/or the levels of all or most of the other forms of A β . In one embodiment, the step can include identifying an agent that reduces A β 42 levels and/or increases A β 39 levels.

With respect to any of the methods for identifying agents that modulate A β levels without substantially altering or affecting the cleavage and/or processing of a presenilin substrate that is other than APP, the identified agents either do not alter the cleavage and/or processing (in particular the presenilin-dependent cleavage and/or processing) of a presenilin substrate, or alter it in a way that it is substantially unchanged. Such alterations can be determined in a number of ways. For example, an alteration of the cleavage and/or processing of the presenilin substrate that is not substantial can be one that generally is not associated with any significant undesired or adverse consequence in a biological context, such as, for example, in a cell, cell medium, tissue or organism. An alteration of the cleavage and/or processing of the presenilin substrate that is not substantial can also be one that is assessed as a difference in the processing and/or cleavage of the substrate, or the levels of a fragment(s) of the presenilin substrate, in test and control samples that is less than about 40%, 35%, 30%, 25% or 20%. In a particular embodiment of the methods, an alteration that is not substantial can be one that is

- 159 -

assessed as a difference in the processing and/or cleavage of the substrate, or the levels of a fragment(s) of the presenilin substrate, in test and control samples that is less than or equal to about 20%.

In the methods for identifying agents that modulate A β levels without
5 substantially altering or affecting the cleavage and/or processing of a presenilin substrate that is other than APP, the presenilin substrate can be, for example, a peptide, polypeptide, protein or fragment(s) thereof that is altered (*e.g.*, proteolytically processed, at least in part) in a presenilin-dependent manner. Thus, for example, in the case of a presenilin substrate that is altered by proteolytic processing of the substrate, if presenilin
10 is absent, or presenilin activity is inhibited or reduced, the proteolytic processing of the presenilin substrate is altered, for example by an alteration in the levels and/or composition of fragments generated from the substrate, relative to the proteolytic processing of the substrate that occurs in the presence of normal (*e.g.*, wild-type) presenilin activity. Exemplary presenilin substrates include, but are not limited to LRP,
15 Notch, TrkB, APLP2, hIre1 α , E-cadherin and Erb-B4.

Thus, in particular embodiments of the methods for identifying agents that modulate A β levels without substantially altering or affecting the cleavage and/or processing of a presenilin substrate that is other than APP, agents are identified that modulate the levels of one or more A β peptides, such as A β 42, without substantially
20 altering or affecting the cleavage and/or processing (in particular, the presenilin-dependent cleavage and/or processing) of Notch, LRP, E-cadherin, Erb-B4, TrkB, APLP2 and/or hIre1 α . Such methods can involve, for example, comparing the levels in test and control samples of Notch nuclear intracellular carboxyl domain (NICD), LRP carboxy terminal fragments (CTFs), E-cadherin intracellular carboxyl domain (ICD),
25 and/or Erb-B4 intracellular carboxyl domain (ICD). In a particular embodiment of the method, the levels of one or more LRP fragments, *e.g.*, LRP-CTFs, in test and control samples are compared. The processing and processing patterns of these presenilin substrates, and characteristic fragments that can be generated therefrom, are described herein.

30 In a particular embodiment of the methods provided herein for identifying an

- 160 -

agent that modulates A β levels without substantially altering the processing and/or cleavage of LRP, the process identifying an agent that does not substantially alter the cleavage of LRP can involve a comparison of the cleavage and/or processing of LRP, and/or the levels of a peptide fragment(s) of LRP, in a sample that has been contacted
5 with the test agent (i.e., test sample) and in a sample that has not been contacted with the test agent (i.e., control sample). The processing or cleavage of an LRP or fragment(s) thereof can be assessed by determining the presence or absence and/or level of one or more fragments of LRP and/or the composition of LRP using, for example, materials and methods described herein. Thus, the LRP composition can be evaluated to determine if
10 any fragment(s) indicative of presenilin-dependent cleavage of LRP or altered presenilin-dependent cleavage of LRP are present and/or the level of any such fragments. Such fragments and compositions are described herein.

In one embodiment, the processing or cleavage of an LRP or fragment(s) thereof is assessed by determining the presence or absence and/or level of an LRP fragment that
15 is cleaved in the presence of a presenilin-dependent activity (presenilin-dependent γ -secretase activity), and thus absent (or present at low levels) in the presence of the presenilin-dependent activity, but that can be detected intact when the presenilin-dependent activity is altered (such that it is eliminated or reduced). In one embodiment, the presence or absence and/or level of an LRP fragment having a molecular weight of
20 between about 25 kD and 15 kD, and, in particular, about 20 kD, is assessed. Typically, the ~ 20 kD fragment is one that is present when an LRP is not cleaved by a presenilin-dependent activity, such as one that occurs in the presence of an inhibitor of a presenilin-dependent activity such as DAPT. In a particular embodiment, the fragment is from a C-terminal portion of LRP, i.e., a CTF. The LRP fragment can be one that contains an
25 amino acid sequence located within the sequence of amino acids 4420-4544 of SEQ ID NO:10. In a further embodiment, the fragment is one that is recognized by an antibody generated against C-terminal amino acids (e.g., the C-terminal 13 amino acids) of LRP, such as, for example, the polyclonal antibody R9377 described herein.

In a particular embodiment of the methods provided herein for identifying agents
30 that modulate A β levels without substantially altering or affecting the cleavage and/or

- 161 -

processing of a presenilin substrate that is other than APP, agents that have been identified as agents that reduce A β 42 levels (*e.g.*, by $\geq 50\%$ at, *e.g.*, 30 μM ; see, for example, EXAMPLE 6) are tested for any effects on presenilin-dependent substrate processing activity by assessing the cleavage and/or processing of LRP in the presence of the A β 42-reducing agent (test sample) and comparing it to negative and positive control samples. In this particular embodiment, LRP processing is assessed by determining the presence or absence, and, if present, the level of an ~ 20 kDa fragment from a C-terminal portion of LRP. The fragment can be detected, for example, using an antibody generated against the C-terminal 13 amino acids of LRP. An A β 42-reducing agent is selected as one that does not substantially alter the cleavage and/or processing of LRP if the level of the ~ 20 kDa fragment of LRP in a sample that had been contacted with the agent (*e.g.*, at 30 μM) is less than about 20% of that in a positive control sample in which presenilin-dependent γ -secretase activity has been inhibited (*e.g.*, using DAPT at ~ 1 μM or 1 mM).

In other embodiments of the methods for identifying or screening for agents that modulate A β levels without substantially altering or affecting the cleavage and/or processing of a presenilin substrate that is other than APP, agents are identified that modulate the levels of one or more A β peptides, such as A β 42, without substantially altering or affecting the cleavage and/or processing (in particular, the presenilin-dependent cleavage and/or processing) of Notch, E-cadherin, Erb-B4, TrkB, APLP2 and/or hIrel α . In particular embodiments, these methods can involve, for example, comparing the levels (and/or presence or absence) in test and control samples of one or more fragments of Notch, E-cadherin and/or Erb-B4 (as well as LRP) or portion(s) thereof. The methods that involved assessing processing of Notch, E-cadherin or Erb-B4 can be conducted, for example, in a manner similar to that described herein for methods that involve assessing LRP processing. Because alteration, such as, for example, inhibition, of the presenilin-dependent cleavage of Notch can result in adverse side effects including, for example, immunodeficiency and anaemia, one embodiment of the methods described herein includes screening for A β -modulating agents that do not substantially alter Notch cleavage and/or processing (in particular, presenilin-dependent processing). Furthermore, non-specific modulation of presenilin and/or presenilin-

- 162 -

dependent activity may affect E-cadherin and/or Erb-B4 processing resulting in adverse side effects and, therefore, in particular embodiments of the methods described herein, agents are identified that modulate A β levels without substantially altering or affecting E-cadherin and/or Erb-B4 processing. In particular embodiments, the method can involve
5 identifying agents that modulate A β levels without substantially altering the cleavage and/or processing of one or more or all of LRP, Notch, E-cadherin and Erb-B4.

(3) Assessment of carboxy-terminal fragments of APP and APP AICD

In addition, other parameters of APP processing may be monitored to determine if
10 the cellular pathway is being altered by an A β modulating agent in a way that may result in adverse side effects. For example, an agent that inhibits γ -secretase may cause the accumulation of high amounts of the carboxy terminal fragment species of APP cleaved by α - or β -secretase. Such fragments may be neurotoxic at high levels. Accumulation of these fragments or the N-terminal fragments produced by α - or β -secretase can be
15 determined by immunoassaying cell lysates with an appropriate antibody prepared to such peptides.

One method provided herein for identifying or screening for agents that selectively modulate A β levels includes steps of contacting a sample containing APP, or portion(s) thereof and α - and/or β -secretase activity with a test agent that modulates A β
20 levels and identifying a test agent as an agent that selectively modulates A β levels if the agent does not substantially alter the level or composition of fragments produced by α - or β -secretase. The agent that modulates A β levels that is used in this method can be any agent known to modulate A β levels. The agent can, for example, be one that is identified by a method described herein which involves contacting a sample containing APP or
25 portion(s) thereof with a test agent and identifying an agent that alters the cleavage of APP that produces one or more A β peptides, the processing of APP and/or the level of one or more A β peptides.

The step of identifying an agent that does not substantially alter the level or composition of fragments produced by α - or β -secretase can be carried out in a number of
30 ways. In general, this process can involve a comparison of the α - and/or β -secretase

- 163 -

cleavage of APP (or portion thereof) that has been contacted with the test agent (i.e., test sample) and of a sample that has not been contacted with the test agent (i.e., control sample). If the level or composition of fragments produced by α - and/or β -secretase cleavage of APP (or portion thereof) in the test and control samples do not differ

5 substantially, then the agent is identified as one that alters the level of one or more A β peptides without substantially altering the α - and/or β -secretase cleavage of APP. The control sample can be the same physical sample as the test sample or a different sample. When the control and test samples are the same, the control is the sample in the absence of test agent.

10 In a particular embodiment, the agent that modulates A β levels that is used in the method is one that modulates the levels of A β 42. In a further embodiment, the agent can be one that selectively modulates the levels of A β 42 relative to A β 40 levels and/or the levels of all or most of the other forms of A β . In a particular embodiment, the agent modulates the levels of A β 42 and A β 39 relative to A β 40 levels and/or the levels of all or
15 most of the other forms of A β . In one embodiment, the agent reduces A β 42 levels and/or increases A β 39 levels.

Another method provided herein for identifying or screening for an agent that selectively modulates A β levels includes steps of contacting a sample containing APP and/or a portion(s) thereof (including, for example one or more A β peptides) and α -
20 and/or β -secretase activity with a test agent and identifying an agent that alters the A β peptide-producing cleavage or processing of the APP (and/or portion(s) thereof), the processing of A β and/or the levels of one or more A β peptides without substantially altering the α - and/or β -secretase cleavage of APP. The process of identifying an agent that selectively modulates one or more A β peptides can be carried out in a number of
25 ways as described herein.

The process of further identifying an agent that does not substantially alter the level or composition of fragments produced by α - or β -secretase cleavage of APP, can also be carried out in a number of ways, as described herein. In general, this process can involve a comparison of the α - and/or β -secretase cleavage of APP (or portion thereof)
30 that has been contacted with the test agent (i.e., test sample) and of a sample that has not

- 164 -

been contacted with the test agent (i.e., control sample). If the level or composition of fragments produced by α - and/or β -secretase cleavage of APP (or portion thereof) in the test and control samples do not differ substantially, then the agent is identified as one that alters the level of one or more A β peptides without substantially altering the α - and/or β -secretase cleavage of APP. The control sample can be the same physical sample as the test sample or a different sample. When the control and test samples are the same, the control is the sample in the absence of test agent.

In this method of identifying an agent that selectively modulates A β levels, the identification of the A β -modulating agent and the determination as to whether the agent alters the levels and/or composition of α - and/or β -secretase cleavage of APP, or portion thereof, can be conducted sequentially or simultaneously. For example, an agent that modulates A β levels can be identified by a difference in the processing of APP or A β , and/or the level of one or more A β peptides in samples contacted with the agent (test sample) and samples not contacted with the agent (control sample). The identified agent can then be separately evaluated for its effects on α - and/or β -secretase cleavage of APP by comparing the α - and/or β -secretase cleavage of APP (or portion thereof) in a sample that has been contacted with the test agent (i.e., test sample) and in a sample that has not been contacted with the test agent (i.e., control sample). In this sequential method, the sample used in the identification of the A β -modulating agent can be of the same or different type relative to the sample used in the determination as to whether the agent alters the α - and/or β secretase cleavage of APP. If the same sample is used, it can contain APP (and/or portion(s) thereof) and an α - and/or β -secretase. If different types of samples are used, the sample used in the identification of the A β -modulating agent can contain APP and/or portion(s) thereof, and the sample used in the determination of alteration in the α - and/or β -secretase cleavage of APP can contain APP and/or portion(s) thereof, and an α - and/or β -secretase.

Alternatively, in a simultaneously performed method, a test sample containing APP (and/or portion(s) thereof), and an α - and/or β -secretase can be contacted with a test agent and the cleavage of APP that produces one or more A β peptides, the processing of APP or A β , and/or the level of one or more A β peptides can be assessed for the test

- 165 -

sample, as can α - and/or β -secretase cleavage of APP be assessed for the same test sample. The A β peptide-producing cleavage or processing of APP, processing of A β and/or levels of A β peptides of the test sample, as well as the α - and/or β -secretase cleavage of APP of the test sample, can be compared to that of a control sample in one
5 step to identify an agent that modulates A β levels without substantially altering the level or composition of fragments produced by α - and/or β -secretase cleavage of APP (or portion thereof).

In the embodiments of the methods provided herein for identifying agents that modulate A β levels without substantially altering or affecting the level or composition of
10 fragments produced by α - and/or β -secretase cleavage of APP (or portion thereof), the fragments produced by α - and/or β -secretase cleavage of APP (or portion thereof) can be detected by any methods known in the art or described herein, for example, using an antibody generated against the amino acids of sAPP α , C83, p3, sAPP β , or C99.

Further studies have demonstrated the production of an intracellular CTF of APP
15 resulting from γ -secretase cleavage, which, in analogy to NICD, is referred to as AICD (APP intracellular domain) (Pinnix, I *et al.* (2001) *J. Biol. Chem* 276:481-487; Sastre, M. *et al.* (2001) *EMBO Reports* 2(9):835-41; Gu, Y *et al.* (2001) *J. Biol Chem.* 276(38): 35235-8). Sequencing has revealed that its N-terminus does not correspond to the expected γ -secretase cleavage after amino acids 40 or 42 of the A β domain. Instead,
20 cleavage occurs between amino acids 49 and 50, close to the cytoplasmic side of the transmembrane domain. Amino acids 49 and 50 of the A β domain correspond to amino acids 720 and 721 of the full length APP protein (see e.g., amino acids 720 and 721 of SEQ ID NOs. 2 and 28). This cleavage is reminiscent of the S3 cleavage of Notch and may thus indicate an analogous function of AICD in signal transduction. Indeed, the
25 cytoplasmic fragment of APP has been shown to form a transcriptionally active complex with Fe65, and Tip60 (Cao, X and Sudhof, T.C. (2001) *Science* 293:115-120). Inhibition of such cleavage may result in unwanted side affects. Thus, in a particular embodiment, a fragment of APP having an N-terminal end that terminates after amino acid 49 of the A β domain (close to the cytoplasmic side of the transmembrane domain) is substantially
30 unchanged in the presence of a test agent when compared to that in the absence of the test

- 166 -

agent.

One method provided herein for identifying or screening for agents that selectively modulate A β levels includes steps of contacting a sample containing APP, or portion(s) thereof and γ -secretase activity with a test agent that modulates A β levels and identifying a test agent as an agent that selectively modulates A β levels if the agent does not substantially alter the level or composition of fragments of APP having an N-terminal end that terminates after amino acid 49 of the A β domain. The agent that modulates A β levels that is used in this method can be any agent known to modulate A β levels. The agent can, for example, be one that is identified by a method described herein which involves contacting a sample containing APP or portion(s) thereof with a test agent and identifying an agent that alters the cleavage of APP that produces one or more A β peptides, the processing of APP and/or the level of one or more A β peptides.

The step of identifying an agent that does not substantially alter the level or composition of fragments of APP having an N-terminal end that terminates after amino acid 49 of the A β domain can be carried out in a number of ways. In general, this process can involve a comparison of the γ -secretase cleavage of APP (or portion thereof) that has been contacted with the test agent (i.e., test sample) and of a sample that has not been contacted with the test agent (i.e., control sample). If the level or composition of fragments produced by γ -secretase cleavage of APP (or portion thereof) with an N-terminal end that terminates after amino acid 49 of the A β domain in the test and control samples do not differ substantially, then the agent is identified as one that alters the level of one or more A β peptides without substantially altering the level or composition of fragments of APP with an N-terminal end that terminates after amino acid 49 of the A β domain. The control sample can be the same physical sample as the test sample or a different sample. When the control and test samples are the same, the control is the sample in the absence of test agent.

In a particular embodiment, the agent that modulates A β levels that is used in the method is one that modulates the levels of A β 42. In a further embodiment, the agent can be one that selectively modulates the levels of A β 42 relative to A β 40 levels and/or the levels of all or most of the other forms of A β . In a particular embodiment, the agent

- 167 -

modulates the levels of A β 42 and A β 39 relative to A β 40 levels and/or the levels of all or most of the other forms of A β . In one embodiment, the agent reduces A β 42 levels and/or increases A β 39 levels.

Another method provided herein for identifying or screening for an agent that
5 selectively modulates A β levels includes steps of contacting a sample containing APP
and/or a portion(s) thereof (including, for example one or more A β peptides) and γ -
secretase activity with a test agent and identifying an agent that alters the A β peptide-
producing cleavage or processing of the APP (and/or portion(s) thereof), the processing
of A β and/or the levels of one or more A β peptides without substantially altering the
10 level or composition of fragments of APP having an N-terminal end that terminates after
amino acid 49 of the A β domain. The process of identifying an agent that selectively
modulates one or more A β peptides can be carried out in a number of ways as described
herein.

The process of further identifying an agent that does not substantially alter the
15 level or composition of fragments of APP having an N-terminal end that terminates after
amino acid 49 of the A β domain, can also be carried out in a number of ways, as
described herein. In general, this process can involve a comparison of the γ -secretase
cleavage of APP (or portion thereof) that has been contacted with the test agent (i.e., test
sample) and of a sample that has not been contacted with the test agent (i.e., control
20 sample). If the level or composition of fragments of APP having an N-terminal end that
terminates after amino acid 49 of the A β domain in the test and control samples do not
differ substantially, then the agent is identified as one that alters the level of one or more
A β peptides without substantially altering the level or composition of fragments of APP
having an N-terminal end that terminates after amino acid 49 of the A β domain. The
25 control sample can be the same physical sample as the test sample or a different sample.
When the control and test samples are the same, the control is the sample in the absence
of test agent.

In this method of identifying an agent that selectively modulates A β levels, the
identification of the A β -modulating agent and the determination as to whether the agent
30 alters the levels and/or composition of fragments of APP having an N-terminal end that

- 168 -

terminates after amino acid 49 of the A β domain, or portion thereof, can be conducted sequentially or simultaneously. For example, an agent that modulates A β levels can be identified by a difference in the processing of APP or A β , and/or the level of one or more A β peptides in samples contacted with the agent (test sample) and samples not contacted with the agent (control sample). The identified agent can then be separately evaluated for its effects on fragments of APP having an N-terminal end that terminates after amino acid 49 of the A β domain by comparing the fragments of APP having an N-terminal end that terminates after amino acid 49 of the A β domain (or portion thereof) in a sample that has been contacted with the test agent (i.e., test sample) and in a sample that has not been contacted with the test agent (i.e., control sample). In this sequential method, the sample used in the identification of the A β -modulating agent can be of the same or different type relative to the sample used in the determination as to whether the agent alters the level and/or composition of fragments of APP having an N-terminal end that terminates after amino acid 49 of the A β domain. If the same sample is used, it can contain APP (and/or portion(s) thereof) and an γ -secretase. If different types of samples are used, the sample used in the identification of the A β -modulating agent can contain APP and/or portion(s) thereof, and the sample used in the determination of alteration in the fragments of APP having an N-terminal end that terminates after amino acid 49 of the A β domain can contain APP and/or portion(s) thereof, and a γ -secretase activity.

Alternatively, in a simultaneously performed method, a test sample containing APP (and/or portion(s) thereof), and a γ -secretase can be contacted with a test agent and the cleavage of APP that produces one or more A β peptides, the processing of APP or A β , and/or the level of one or more A β peptides can be assessed for the test sample, as can fragments of APP having an N-terminal end that terminates after amino acid 49 of the A β domain be assessed for the same test sample. The A β peptide-producing cleavage or processing of APP, processing of A β and/or levels of A β peptides of the test sample, as well as the fragments of APP having an N-terminal end that terminates after amino acid 49 of the A β domain, can be compared to that of a control sample in one step to identify an agent that modulates A β levels without substantially altering the level or composition of fragments of APP having an N-terminal end that terminates after amino

- 169 -

acid 49 of the A β domain (or portion thereof).

In the embodiments of the methods provided herein for identifying agents that modulate A β levels without substantially altering or affecting the level or composition of fragments of APP having an N-terminal end that terminates after amino acid 49 of the A β domain, the APP fragments having an N-terminal end that terminates after amino acid 49 of the A β domain can be detected by any methods known in the art or described herein, for example, using an antibody generated against the C-terminal amino acids of APP. The C-terminal amino acids may include any amino acid C-terminal to amino acid 49 of the A β domain or any amino acid C-terminal to amino acid 720 of full length APP.

10 E. Systems

There are a number of kits, combinations and systems that can be used in performing the various methods provided herein. Such methods include methods for assessing presenilin activity, methods for identifying candidate agents for treatment or prophylaxis of a disease or disorder associated with an altered presenilin, methods for identifying or screening for agents that modulate A β levels and methods for identifying or screening for agents for treatment or prophylaxis of a disease or disorder characterized by and/or associated with altered A β levels and/or processing of APP, including for example, diseases associated with amyloidosis.

Kits, combinations and systems are also provided herein. Such kits, combinations and/or systems can include, for example, a cell(s) (and/or lysates, extracts, medium and membranes from the cell(s)) exhibiting APP (altered and/or wild-type as well as portion(s) of APP) expression and processing, one or more presenilins (altered and/or wild-type as well as portion(s) of presenilins) expression and processing, and/or one or more presenilin substrates (altered and/or wild-type as well as portion(s) of presenilin substrates), including, for example, LRP, Notch, E-cadherin and Erb-B4. The cells of the system can be isolated cells or cell cultures that endogenously express such protein(s) or can recombinantly express such proteins as described above with respect to the methods for identifying agents. Systems in which the cells recombinantly express the proteins can be such that the cells are isolated cells or cell cultures or are contained within an animal, in particular, a non-human animal, *e.g.*, a non-human mammal. Many

- 170 -

examples of such cells are described herein and known in the art.

The kits, combinations and/or systems provided herein can include antibodies and/or fragment(s) thereof specifically reactive to particular A β peptides. For example, a system can include antibodies specifically reactive to A β 42 versus one or more other A β peptides, and in particular, A β 40. A β 42 selective-antibodies are provided herein. Such antibodies can be made by the methods described herein, including, for example, by immunization with a peptidyl sequence of MVGGVIA, and by recombinant methods. One such antibody (and/or fragment(s) thereof) includes the sequence of amino acids 1-95 of SEQ ID NO:12 and/or 1-97 of SEQ ID NO: 14. A kit, combination or system can include cells that produce any such antibody (and/or fragment(s) thereof). For example, such a cell could contain nucleic acid containing the sequence of nucleotides set forth as nucleotides 1-285 of SEQ ID NO: 11 and/or the sequence of nucleotides set forth as nucleotides 1-291 of SEQ ID NO: 13.

The kits, combinations and/or systems provided herein can include detection antibodies (and/or fragment(s) thereof) designed to be reactive to more than one species of A β . In one example, the antibodies that are reactive to a sequence on the N-terminus of A β , such as, for example amino acids 1-12 of A β . Such antibodies (and/or fragment(s) thereof) are provided herein and include antibodies containing one or both of the amino acids 1-100 of SEQ ID NO: 16 and 1-98 of SEQ ID NO: 18. A kit, combination or system can include cells that produce any such antibody (and/or fragment(s) thereof). For example, such a cell could contain nucleic acid containing the sequence of nucleotides set forth as nucleotides 1-300 of SEQ ID NO: 15 and/or the sequence of nucleotides set forth as nucleotides 1-294 of SEQ ID NO: 17. The detection antibody is generally conjugated to a detectable label, such as, for example alkaline phosphatase, and the presence or absence of antibody binding can be determined by luminescence of a substrate that is detected by a change in light emitted in the presence of alkaline phosphatase, such as, for example, CDP-Star chemiluminescence substrate (Tropix, Inc.).

One system provided herein can be used, for example, in assessing presenilin activity. In a particular embodiment, the system includes a source of presenilin activity, a

- 171 -

source of LRP (and/or portion(s) thereof) protein and a reagent for determining LRP protein composition. In one embodiment, the source of presenilin activity can be, for example, a standard or control used in a method of assessing presenilin activity. In another embodiment, the source of presenilin activity can be the activity that is being
5 assessed. An example of a reagent for determining LRP protein composition is an antibody (and/or fragment(s) thereof) that recognizes a fragment of LRP generated by a presenilin-dependent activity, *e.g.*, presenilin-dependent γ -secretase or a LRP fragment that occurs in the absence of such activity. Such fragments include LRP-CTF, and, in particular an ~ 20 kD fragment of LRP. In one embodiment, the system includes an anti-
10 LRP antibody prepared to the carboxyl-terminal 13 amino acid peptide of LRP (C-GRGPEDEIGDPLA). In a particular embodiment, the system includes the anti-LRP polyclonal antibody (R9377) described herein (see, *e.g.*, the EXAMPLES). Some systems can also contain sources of other presenilin substrates, *e.g.*, Notch, Erb-B4 and E-cadherin) and reagents, such as antibodies and/or fragment(s) thereof, that are reactive
15 to Notch intracellular domain (NICD), E-cadherin intracellular domain, or Erb-B4 intracellular domain.

One embodiment of a system or kit for use in identifying agents that modulate $A\beta$ levels provided herein contains a reagent for assessing cleavage of APP that produces one or more $A\beta$ peptides, APP processing, $A\beta$ processing and/or $A\beta$ levels and a reagent
20 for assessing cleavage and/or processing (in particular, presenilin-dependent processing) of a presenilin substrate. In a particular embodiment, the presenilin substrate is LRP and/or portion(s) thereof. Such reagents are described and provided herein. For example, reagents for assessing $A\beta$ levels include antibodies and/or fragments thereof such as antibodies that specifically react with $A\beta_{42}$, for example an antibody or fragment(s)
25 thereof containing the sequence of amino acids 1-95 of SEQ ID NO:12 and/or 1-97 of SEQ ID NO: 14. Another example of an antibody that can be used in assessing $A\beta$ levels is an antibody that recognizes most or all forms of $A\beta$. One example is an antibody (and/or fragment(s) thereof) containing one or both of the amino acids 1-100 of SEQ ID NO: 16 and 1-98 of SEQ ID NO: 18. An example of a reagent for determining LRP
30 protein composition in assessing LRP cleavage and/or processing is an antibody (and/or

- 172 -

fragment(s) thereof) that recognizes a fragment of LRP generated by a presenilin-dependent activity, *e.g.*, presenilin-dependent γ -secretase or a LRP fragment that occurs in the absence of such activity. Such fragments include LRP-CTF, and, in particular an ~20 kD fragment of LRP. In one embodiment, the system includes an anti-LRP antibody
5 prepared to the carboxyl-terminal 13 amino acid peptide of LRP (C-GRGPEDEIGDPLA). In a particular embodiment, the system includes the anti-LRP polyclonal antibody (R9377) described herein (see, *e.g.*, the EXAMPLES). Some systems can also contain reagents such as antibodies and/or fragment(s) thereof that are reactive to Notch intracellular domain (NICD), E-cadherin intracellular domain, or Erb-
10 B4 intracellular domain.

F. Methods of Identifying Agents for the Treatment of a Disease or Disorder

Provided herein are methods for identifying candidate agents for the treatment or prophylaxis of diseases and disorders associated with or characterized by altered APP processing, A β production, catabolism, processing and/or levels. Disease models are a
15 valuable tool for the discovery and testing of treatment agents. Such disease models may be cellular or organismal and may be produced by methods known to those of skill in the art and described herein.

1. Cell models

Cell models for the identification and testing of agents for the treatment of
20 diseases and disorders characterized by altered A β peptide levels are provided herein. Suitable cell lines include human and animal cell lines, such as the 293 human kidney cell line, neuroglioma cell lines, neuroblastoma cell lines, HeLa cells, primary endothelial cells, primary fibroblasts or lymphoblasts, primary mixed brain cells (including neurons, astrocytes, and neuroglia), Chinese hamster ovary (CHO) cells, and
25 the like.

In a particular embodiment, mixed brain cell cultures from transgenic mice (*e.g.*, Tg2576 transgenic mice) are provided. Such primary cultures can mimic an *in vivo* system more closely than engineered cell lines. Primary mixed brain cultures can be established by any method known to those of skill in the art or described herein.
30 Generally, primary mixed brain cultures can be produced by dissecting 17 day old mouse

- 173 -

embryos utilizing a stereo scope, obtaining brain tissue and dissociating with papain, then culturing cells by standard procedures for primary neuronal cultures.

Primary cell cultures can be obtained from any host, in a particular embodiment, a non-human host, including but not limited mice, rabbits, monkeys, apes, etc. which
5 naturally express APP or any one or combination of isoforms or fragments of APP. The primary cultures can comprise cells that express wild type versions or isoforms of APP or mutant versions. The cells can over express the protein as well.

Alternatively, engineered cell lines may be used. Cells may contain recombinant DNA that when expressed, result in altered production, degradation or clearance of A β
10 peptides or altered expression of APP, such as by replacing or modifying the promoter region or other regulatory region of the endogenous gene. Such a cell can be produced by introduction of heterologous or homologous nucleic acid into the cell using methods known in the art and described herein. In a particular embodiment, the cell is a recombinant cell that expresses the protein(s) as heterologous protein(s). Such cells may
15 overexpress or mis-express the heterologous protein(s). For example, a recombinant cell may be one that endogenously expresses the protein(s) and also has been transfected with additional copies of nucleic acid encoding the protein(s). Alternatively, the host cell used in the generating the recombinant cell may be one that endogenously expresses little to none of the protein(s) of interest or one in which such proteins have been eliminated
20 (*e.g.*, through gene knock-out methods or by inhibition with an agent that does not inhibit the activity of the heterologous protein(s)). In a particular embodiment, cell lines capable of expressing APP variants with altered A β peptide levels are provided. Such variants can include those having one or several amino acid substitutions directly amino-terminal of the A β cleavage site. For example, APP DNA bearing a double mutation (Lys⁵⁹⁵-
25 >Asn⁵⁹⁵ and Met⁵⁹⁶->Leu⁵⁹⁶) found in a Swedish FAD family produce approximately six-to-eight fold more A β than cells expressing normal APP. Exemplary clones and vectors for APP include but are not limited ATCC accession numbers 40305, 40347, 78397, 78510, 78510D, 86195.

Cells or less differentiated precursor cells may be stably or transiently transfected
30 with purified or recombinant protein(s) in vitro or in an organism. In vitro transfection is

- 174 -

followed by cell expansion through culturing prior to use. Cells from a known cell line are preferred, such as from neuroblastoma SH-SY5Y cells, pheochromocytoma PC12 cells, neuroblastoma SK-N-BE(2)C cells, human SK-N-MC neuroblastoma cells, SMS-KCNR cells, human LAN-5 neuroblastoma cells, human GI-CA-N neuroblastoma cells, 5 human neuroblastoma cells, mouse Neuro 2a (N2A) neuroblastoma cells and/or human IMR 32 neuroblastoma cells. Exemplary cell lines include human embryonic kidney 293 (HEK 293) ATCC accession number CRL-1573, CHO (including CHO and CHO-K1 (accession number CCL-61)), LTK⁻, N2A (accession number CCL-131), H6, and HGB. The generation, maintenance and use of such cell lines is well known.

10 Suitable cells include mammalian cell lines, typically human cell lines that are commercially available for example from the American Type Tissue Culture Collection (ATCC), Rockville, Maryland, 20852. Exemplary cells include CHO cells expressing human APP751 from a vector containing the gene encoding APP751, human mutant APP751 (V717F) from a vector containing a gene encoding APP751 (V717F), or a 15 combination thereof and can be cultured in standard cell culture media supplemented with 10% fetal calf serum and optionally with antibiotics and fungicides such as 100 U/mL penicillin/streptomycin. Other suitable cells include human neuroglioma cells HS683 that express APP695, APP751, APP770 or a combination thereof from a vector containing a gene encoding for the respective protein or partial protein. Additionally, a 20 human neuroblastoma cell line SH-SY5Y described in T. Yamazaki and Y. Ihara (1998) *Neurobiology of Aging* 19:S77-S79 or other cell that secretes large amounts of A β into the medium without A β transfection can also be used.

An exemplary transformed human embryonic kidney cell line is the human 293 cell line, ATCC accession number CRL-1573. Other suitable cells include CRL-1721 25 and CCL-92 and those listed in the catalogue from the Indiana Alzheimer Disease Center National Cell Repository of Indiana University - Purdue University Indianapolis, 425 University Blvd., Indianapolis, IN 46202-5143, which is incorporated by reference herein in its entirety.

Additionally, primary cell cultures, immortalized cell lines, or stem cells 30 (embryonic or adult) induced to express A β proteins or peptides can be used. In one

- 175 -

embodiment, cells that are not terminally differentiated can be induced to express neuronal characteristics. Such cells can be induced for example by exposing them to a growth factor, cytokine, hormone, neural inducing media or combination thereof.

2. Animal models

5 Animal models for the identification and testing of agents for the treatment of diseases and disorders characterized by altered A β peptide levels are provided herein. Transgenic animal models and animals, such as rodents, including mice and rats, cows, chickens, pigs, goats, sheep, monkeys, including gorillas, and other primates, are provided herein. In particular, transgenic non-human animals that contain recombinant
10 DNA that when expressed, result in altered production, degradation and/or clearance of A β peptides or altered expression of APP, such as by replacing or modifying the promoter region or other regulatory region of the endogenous gene are provided. Such an animal can be produced by promoting recombination between endogenous nucleic acid and an exogenous gene of interest that could be over-expressed or mis-expressed, such as
15 by expression under a strong promoter, via homologous or other recombination event.

 Transgenic animals can be produced by introducing the nucleic acid using any known method of delivery, including, but not limited to, microinjection, lipofection and other modes of gene delivery into a germline cell or somatic cells, such as an embryonic stem cell. Typically the nucleic acid is introduced into a cell, such as an embryonic stem
20 cell (ES), followed by injecting the ES cells into a blastocyst, and implanting the blastocyst into a foster mother, which is followed by the birth of a transgenic animal. Generally introduction of a heterologous nucleic acid molecule into a chromosome of the animal occurs by a recombination between the heterologous nucleic acid of interest and endogenous nucleic acid. The heterologous nucleic acid can be targeted to a specific
25 chromosome.

 In some instances, knockout animals can be produced. Such an animal can be initially produced by promoting homologous recombination between a gene of interest in its chromosome and the corresponding exogenous gene of interest that has been rendered biologically inactive (typically by insertion of a heterologous sequence, *e.g.*, an
30 antibiotic resistance gene). In one embodiment, this homologous recombination is

- 176 -

performed by transforming embryo-derived stem (ES) cells with a vector containing the insertionally inactivated gene of interest, such that homologous recombination occurs, followed by injecting the ES cells into a blastocyst, and implanting the blastocyst into a foster mother, followed by the birth of the chimeric animal ("knockout animal") in which a gene of interest has been inactivated (see Capecchi, *Science* 244:1288-1292 (1989)).

5 The chimeric animal can be bred to produce homozygous knockout animals, which can then be used to produce additional knockout animals. Knockout animals include, but are not limited to, mice, hamsters, sheep, pigs, cattle, and other non-human mammals. For example, a knockout mouse is produced. The resulting animals can serve as models of

10 specific diseases that are the result of or exhibit altered-expression of a polypeptide involved in neurodegenerative disorders. Such knockout animals can be used as animal models of such diseases *e.g.*, to screen for or test molecules for the ability to treat or prevent such diseases or disorders.

Other types of transgenic animals also can be produced, including those that over-

15 express a polypeptide involved in neurodegenerative disorders. Such animals include "knock-in" animals that are animals in which the normal gene is replaced by a variant, such a mutant, an over-expressed form, or other form. For example, one species', such as a rodent's endogenous gene can be replaced by the gene from an other species, such as from a human. Animals also can be produced by non-homologous recombination into

20 other sites in a chromosome; including animals that have a plurality of integration events.

After production of the first generation transgenic animal, a chimeric animal can be bred to produce additional animals with over-expressed or mis-expressed polypeptides involved in neurodegenerative disorders. Such animals include, but are not limited to, mice, hamsters, sheep, pigs, cattle and other non-human mammals. The resulting

25 animals can serve as models of specific diseases that are the result of or exhibit over-expression or mis-expression of a polypeptide involved in neurodegenerative disorders. Such animals can be used as animal models of such diseases *e.g.*, to screen for or test molecules for the ability to treat or prevent such diseases or disorders. In a specific embodiment, a mouse with over-expressed or mis-expressed APP is produced.

30 One useful non-human animal model harbors a copy of an expressible transgene

- 177 -

sequence which encodes the Swedish mutation of APP (Asp595-leu596). US Patent Nos. 5,612,486 and 5,850,003, incorporated herein by reference, disclose a transgenic rodent having a diploid genome comprising a transgene encoding a heterologous APP polypeptide having the Swedish mutation wherein the amino acid residues at positions
5 corresponding to positions 595 and 596 in human APP695 are asparagine and leucine, respectively. The transgene is expressed to produce a human APP polypeptide having the Swedish mutation. The polypeptide is processed in a sufficient amount to be detectable in a brain homogenate of the transgenic rodent. The sequence generally is expressed in cells which normally express the naturally-occurring endogenous APP gene (if present).
10 Murine and hamster models are suitable for this use. Such transgenes typically comprise a Swedish mutation APP expression cassette, in which a linked promoter and, preferably, an enhancer drive expression of structural sequences encoding a heterologous APP polypeptide comprising the Swedish mutation.

Other suitable animal models include the transgenic mouse disclosed in US
15 Patent No. 5,387,742. This transgenic mouse contains a DNA sequence with a nerve tissue specific promoter and a DNA sequence which encodes a β -amyloid precursor protein selected from the group consisting of A751 and A770. The promoter and DNA sequence which encodes the precursor protein are operatively linked to each other and integrated in the genome of the mouse and expressed to form β -amyloid protein deposits
20 in the brain of the mouse.

Still other transgenic animal models for the identification and testing of agents for the treatment of disease and disorders characterized by altered A β peptide levels include those described in US Patent Nos. 5,811,633; 6,037,521; 6,184,435; 6,187,992; 6,211,428; and 6,340,783, all of which are incorporated by reference, transgenic mouse
25 models Tg 2576; APPSWE mouse, K670N, M671L, and other models including APP(V717F), APP(K670N, M671L and V717F), PS-1 M146L, PS-1 M146V, APPSWE + PS A246E (reviewed by Emilien, *et al.*, (2000) *Arch. Neuro.* 57: 176-81).

3. Evaluation of models and identification and testing of agents for the treatment of diseases and disorders

30 Cell and animal models of diseases and disorders involving A β misregulation

- 178 -

described herein have a number of uses. For example, by evaluating the cellular or organismal phenotypes associated with the altered expression of proteins involved in A β regulation in the cells/organisms and correlating such phenotypes with specific cellular molecules and processes, the disease/disorder models can be used in elucidating the mechanisms underlying A β misregulation in a cell as well as in dissecting processes and pathways involved in A β regulation. In addition, by evaluating the effects of test agents or candidate therapeutic agents on A β levels and the phenotypic manifestations of the model cells/organisms, the models can be used in screening agents and testing candidate agents for the treatment of diseases and disorders that involve A β misregulation.

10 In the methods for identifying agents for the treatment or prophylaxis of a disease or disorder, any sample containing an altered test protein, and/or portion(s) thereof, and APP, and/or portion(s) thereof, wherein the altered protein is associated with altered A β 42 production, catabolism, processing and/or A β 42 levels may be used. Such samples can include, for example any cell, cell extract, cell model, organism or animal model

15 described herein. The cell, organism or animal may be one that contains an altered APP, APP processing activity, or A β processing activity and/or expresses altered A β levels such as, for example, the cell and animal models described above. The altered APP, APP processing activity, A β processing activity, or A β level can be one that is altered relative to a wild-type. Typically, a wild-type protein, such as, for example, APP, APP

20 processing enzyme or A β processing enzyme can be one that is encoded by a predominant allele in a population or any allele that is not associated with disease or a pathogenic condition. A wild-type APP, APP processing enzyme or A β processing enzyme can be one that occurs in an organism that exhibits normal APP and/or A β processing patterns. The altered APP, APP processing enzyme or A β processing enzyme

25 can be a mutant or can be, for example, one that is encoded by a nucleic acid linked to Alzheimer's disease. For example, the altered enzyme activity may include any one or more of the at least 60 mutations in human PS1 and the at least two mutations in human PS2 that have been genetically linked to early onset familial Alzheimer's disease (FAD). Exemplary presenilins with altered activity include FAD-associated mutant forms of PS1

30 and PS2 that give rise to an increased accumulation of A β 42 in AD patients and

- 179 -

transfected cell lines and transgenic animals in which they are expressed. Included among such mutations are the PS2 FAD mutation N141I (Volga German FAD mutant) and the PS1 FAD mutation M146L. Examples of diseases associated with an altered APP, APP processing activity, A β , and/or A β processing activity for which the methods
5 provided herein can be used to identify candidate therapeutic or prophylactic agents include, but are not limited to, amyloidosis-associated diseases and neurodegenerative diseases. In a particular aspect, the disease is Alzheimer's Disease.

In one method for testing an agent for use in the treatment of a disease or disorder, the test agent is one that is already known to modulate the level of one or more
10 particular A β peptides. Thus, in one embodiment of this method, a disease model is contacted with a test agent that modulates the level of an A β peptide, and a test agent is identified as an agent for the treatment of a disease or disorder if the test agent at least partially reverses or reduces, ameliorates or eliminates a disease trait or phenotype exhibited by a model cell or organism, or that tends to restore APP processing and/or A β
15 processing or levels to compensate for disease-associated abnormalities in A β levels. In general, the step of identifying a test agent that at least partially reverses or reduces, ameliorates or eliminates a disease trait or phenotype exhibited by a model cell or organism, or that tends to restore APP processing and/or A β processing or levels can involve a comparison of the disease trait or phenotype and/or APP processing and/or A β
20 processing or levels in a model that has been contacted with the test agent (i.e., test model) and in a model that has not been contacted with the test agent (i.e., control model). If the disease trait or phenotype and/or APP processing and/or A β processing or levels in the test and control models differs, then the test agent is identified as a candidate agent for the treatment and/or prophylaxis of a disease or disorder. In such an
25 embodiment, both the test and control model express the disease trait or phenotype in the absence of the test agent. In another embodiment, the control model or sample is a wild type model or sample. In such an embodiment, the step of identifying a candidate agent includes comparing the disease trait or phenotype and/or A β production, catabolism, processing and/or A β levels in a test sample that has been contacted with the test agent
30 and a positive control sample and identifying an agent as a candidate agent A β

- 180 -

production, catabolism, processing and/or A β levels if A β production, catabolism, processing and/or A β levels in the test and control samples is substantially similar

The agent that modulates the level of an A β peptide that is used in this method can be one that was identified by any of the processes described herein. For example, the agent may be one that was identified by a process involving contacting a sample containing APP or portion(s) thereof with a test agent and identifying an agent that alters the cleavage of APP that produces one or more A β peptides, the processing of APP and/or the level of one or more A β peptides.

The agent that modulates the level of an A β peptide that is used in this method can be one that was identified as having a particular selectivity. Methods of assessing the selectivity of an A β modulating agent are provided herein. In a particular embodiment the agent that selectively modulates A β levels can be one that does not substantially alter the level of one or more A β peptides other than the A β peptide that is modulated by the test agent. In a particular embodiment, the agent that modulates A β levels that is used in the method is one that modulates the levels of A β 42. In a further embodiment, the agent can be one that selectively modulates the levels of A β 42 relative to A β 40 levels and/or the levels of all or most of the other forms of A β . In a particular embodiment, the agent modulates the levels of A β 42 and A β 39 relative to A β 40 levels and/or the levels of all or most of the other forms of A β . In one embodiment, the agent reduces A β 42 levels and/or increases A β 39 levels. In a particular embodiment, the agent that reduces A β 42 levels does not substantially alter the levels of non-APP substrate cleavage/processing activity of presenilin, such as LRP and/or other substrates provided herein. In another embodiment the agent that reduces A β 42 levels does not substantially alter the levels of A β 40 or the non-APP substrate cleavage/processing activity of presenilin.

In other embodiments, agents that have not previously been screened for their ability to modulate the level of one or more particular A β peptides may be screened in cellular and organismal disease model systems. An agent can be identified as an agent that alters the cleavage of APP that produces one or more A β peptides, the processing of APP or A β , and/or the level of one or more A β peptides. In one embodiment, an alteration results in the restoration of APP processing and/or A β processing or levels to

- 181 -

compensate for disease-associated abnormalities in A β levels. At the same time, the agent can be identified as an agent that at least partially reverses or reduces, ameliorates or eliminates a disease trait or phenotype exhibited by a model cell or organism. The process of identifying an alteration in APP processing, A β processing and A β levels can
5 be carried out in a number of ways as described herein.

The selectivity of the agent may also be assessed in the disease model system. Any methods of assessing the selectivity of an A β modulating agent provided herein may be used. In a particular embodiment the agent that selectively modulates A β levels does not substantially alter the level of one or more A β peptides other than the A β peptide that
10 is modulated by the test agent. In a particular embodiment, the agent that modulates A β levels that is used in the method is one that modulates the levels of A β 42. In a further embodiment, the agent can be one that selectively modulates the levels of A β 42 relative to A β 40 levels and/or the levels of all or most of the other forms of A β . In a particular embodiment, the agent modulates the levels of A β 42 and A β 39 relative to A β 40 levels
15 and/or the levels of all or most of the other forms of A β . In one embodiment, the agent reduces A β 42 levels and/or increases A β 39 levels. The modulation of a particular A β peptide by the agent can be identified by any of the methods described herein. In general, the modulation of a particular A β peptide by the agent can be identified by a detectable difference in the levels of the A β peptide in the model cell or organism contacted with
20 the agent (test model) and model cells or organisms not contacted with the agent (control models). The agent is one that selectively modulates the levels of the particular A β peptide if any difference (including, for example, absolute and/or percentage difference) in the levels of one or more other A β peptides in model contacted with the agent and model not contacted with the agent is less than the difference (including, for example,
25 absolute and/or percentage difference) in the levels of the particular A β peptide in test and control models. In particular embodiments, the extent to which the agent alters the levels of one or more other A β peptides (i.e., the peptides that are not targeted for modulation) is less than about 40%, 35%, 30%, 25%, or 20%. In one embodiment, the extent to which the agent alters the levels of one or more other A β peptides (i.e., the
30 peptides that are not targeted for modulation) is less than 20%. Any modulation of the

- 182 -

level of the one or more other A β peptides (i.e., the peptides that are not targeted for modulation) that is not a substantial alteration is one that is generally not associated with any significant undesired or adverse consequence in the model cell or organism.

In a particular embodiment, agents that more specifically or selectively modulate A β levels can be identified in a disease model using methods provided herein that involve identifying agents that modulate A β levels without substantially altering or affecting non-APP substrate cleaving/processing activity of presenilin. In one embodiment, the agent that reduces A β 42 levels does not substantially alter the levels of non-APP substrate cleavage/processing activity of presenilin, such as LRP and/or other substrates provided herein. In another embodiment the agent that reduces A β 42 levels does not substantially alter the levels of A β 40 or the non-APP substrate cleavage/processing activity of presenilin. The process of further identifying an agent that does not substantially alter the cleavage of a presenilin substrate (other than APP), or portion(s) thereof, can be carried out by any of the methods described herein. In general, this process can involve a comparison of the cleavage and/or processing (in particular, the presenilin-dependent cleavage and/or processing) of a presenilin substrate (or portion(s) thereof) other than APP, and/or the levels of a peptide fragment(s) of the presenilin substrate, in a model cell or cells within a model organism that has been contacted with the test agent (i.e., test model) and in a model cell or cells within a model organism that has not been contacted with the test agent (i.e., control model). If the cleavage and/or processing of the presenilin substrate that is other than APP and/or the substrate fragment(s) levels of the test and control samples do not differ substantially, then the agent is identified as one that alters the level of one or more A β peptides without substantially altering the cleavage and/or processing of the presenilin substrate, or portion(s) thereof, that is other than APP. The control model can be the same physical model as the test model or a different model. When the control and test models are the same, the control is the model in the absence of test agent.

G. Methods for Treating or Preventing Diseases or Disorders

Methods provided herein for identifying or screening for agents that modulate A β levels and for candidate agents for the treatment or prophylaxis of disease are useful in

- 183 -

the discovery of particular agents for treating diseases and disorders involving or characterized by altered A β production, catabolism, processing and/or levels. Such diseases include, but are not limited to, diseases involving or associated with amyloidosis and neurodegenerative diseases. One example of such a disease is Alzheimer's disease.

5 Provided herein are methods for treating or preventing diseases and disorders involving or characterized by altered A β production, catabolism, processing and/or levels. The methods are particularly suitable for the treatment or prevention of disease because they are designed to selectively modulate A β levels, and in particular, the level of A β 42 and/or A β 39, in order to avoid possible side-effects that non-specific
10 modulation of A β can be associated with as described herein. Such methods can include a step of administering to a subject having such a disease or disorder or predisposed to such a disease or disorder an agent that modulates the cleavage of APP that produces one or more A β peptides, the processing of APP, the processing of A β and/or the level of one or more A β peptides. In one embodiment of the methods, the agent being administered is
15 one that modulates the cleavage of APP that produces one or more A β peptides, the processing of APP, the processing of A β and/or the level of one or more A β peptides such that A β 42 levels are modulated. The level of A β 42 can be modulated to a greater extent than the level of one or more other A β peptides, in particular, A β 40, is modulated, or without substantially altering the level of one or more other A β peptides, in particular
20 A β 40. In a particular embodiment, A β 42 levels are reduced.

 In another embodiment, the agent being administered is one that modulates the cleavage of APP that produces one or more A β peptides, the processing of APP, the processing of A β and/or the level of one or more A β peptides such that A β 39 levels are modulated. The level of A β 39 can be modulated to a greater extent than the level of one
25 or more other A β peptides, in particular, A β 40, or without substantially altering the level of one or more other A β peptides, in particular A β 40. In a particular embodiment, the agent increases the level of A β 39. The agent can be one that modulates the levels of A β 42 and A β 39 to a greater extent than the level of one or more other A β peptides, in particular, A β 40, or without substantially altering the levels of one or more other A β
30 peptides, such as, for example, A β 40. In one embodiment, the levels of A β 42 are

- 184 -

reduced and the levels of A β 39 are increased.

In another embodiment of the methods, the agent being administered is one that modulates the cleavage of APP that produces one or more A β peptides, the processing of APP, the processing of A β and/or the level of one or more A β peptides without
5 substantially altering one or more presenilin-dependent activities other than the presenilin-dependent processing of APP. In a further embodiment, the agent being administered is one that modulates the cleavage of APP that produces one or more A β peptides, the processing of APP, the processing of A β and/or the level of one or more A β peptides without substantially altering the cleavage and/or processing of a presenilin
10 substrate and/or portion(s) thereof (*e.g.*, Notch, E-cadherin, Erb-B4, and portion(s) thereof) that is other than APP. In another embodiment, the agent being administered is one that modulates the cleavage of APP that produces one or more A β peptides, the processing of APP, the processing of A β and/or the level of one or more A β peptides without substantially altering the cleavage and/or processing of LRP and/or portion(s)
15 thereof. In particular of these embodiments, the levels of A β 42 and/or A β 39 are modulated, such as, for example, as follows: the levels of A β 42 and/or A β 39 are modulated to a greater extent than the levels of other A β peptides, such as, *e.g.*, A β 40; the levels of A β 42 and/or A β 39 are modulated without substantially altering the level of one or more other A β peptides, such as, *e.g.*, A β 40. In particular embodiments of these
20 methods, the level of A β 42 is reduced and/or the level of A β 39 is increased.

H. Methods of Modulating A β

Provided herein are methods for modulating A β levels. In a particular embodiment, the methods are for selectively modulating A β levels. The methods can be practiced to modulate A β levels in any sample. Examples of samples in which A β levels
25 may be modulated include, but are not limited to, cells, tissues, organisms, lysates, extracts and membrane preparations of cells and cell-free samples, such as, for example, samples containing APP and/or portion(s) thereof.

Modulation of A β can be, for example, any alteration or adjustment that results in a change in A β levels, including but not limited to, alteration of A β levels in the cell
30 cytoplasm, intracellular organelles, cell membranes, extracellular medium, tissue, body

- 185 -

fluid and/or levels of secreted A β . Modulation of A β can involve an alteration in APP (and/or portion(s) thereof) cleavage or processing, A β cleavage or processing and/or any combination thereof. Altered APP cleavage or processing and/or altered A β cleavage or processing may be the result of an alteration in any cell, organelle, enzyme, protein, and/or factor that facilitates or participates in APP cleavage or processing and/or A β cleavage or processing. Cells, organelles, enzymes, proteins and factors that facilitate or participate in APP cleavage or processing and/or A β cleavage or processing may include, but are not limited to microglial cells, proteases, such as secretases, including α , β , and γ -secretases, peptidases, presenilins, degradatory enzymes, including insulin-degrading enzyme (IDE), neprilysin, plasmin, uPA/tPA, endothelin converting enzyme-1, matrix metalloproteinase-9, and proteasome, cell surface receptors, including scavenger receptor A, the receptor for advanced glycation endproducts (RAGE), and the low-density lipoprotein receptor-related protein (LRP). Modulation of A β can also involve an alteration in receptor-mediated clearance and/or uptake into organelles capable of processing A β for degradation, including, for example, endosomes and lysosomes. Modulation of A β levels may thus involve modulating the level, functioning and/or activity of one or more cells, organelles, enzymes, proteins, and/or factors involved in modulating A β production, catabolism, processing and/or clearance.

Modulation of A β levels can be, for example, a complete or nearly complete elimination of the production of one or more forms of A β , a reduction in the production of one or more forms of A β , or an increase in the production of one or more forms of A β . A modulation of A β can also be an increase in clearance and/or degradation of one or more forms of A β , or a decrease in the clearance and/or degradation of one or more forms of A β . Modulation of A β can further be an alteration in the levels of different A β peptides relative to one another or to the total A β . Thus, for example, the ratio of a particular A β peptide to the total A β in a sample can be altered in modulation of A β . A modulation of A β can also be an increase in one or more forms of A β concurrent with a decrease in one or more other forms of A β .

In particular methods for modulating A β provided herein, the cleavage of APP that produces one or more A β peptides, the processing of APP, the processing of A β

- 186 -

and/or the levels of A β is/are modulated in a manner such that A β levels are modulated while avoiding substantial or significant alterations in other processes, activities, mechanisms and/or compositions that are not necessary to modulate in order to modulate A β levels. Such modulation can be a selective or specific modulation of A β levels.

5 In one embodiment, the method selectively modulates the level of particular A β peptides, for example one A β peptide or two A β peptides. In a particular embodiment, the method includes a step of modulating the cleavage of APP that produces one or more A β peptides, the processing of APP, the processing of A β and/or the levels of A β such that the level of A β 42 is modulated to a greater extent than the level of one or more other
10 A β peptides, such as, *e.g.*, A β 40 (or an A β peptide having a C-terminal end that terminates before amino acid 40, or an A β with an N-terminus cleaved after amino acid 49 (close to the cytoplasmic side of the transmembrane domain)) is modulated. The level of A β 42 can be modulated without substantially altering the level of one or more other A β peptides, such as, *e.g.*, A β 40. In a particular embodiment of these methods, the level
15 of A β 42 is reduced; in other embodiments, level of A β 42 is increased. In another particular embodiment, the level of A β 39 (or the level of one or more A β peptides having a C-terminal end that terminates before amino acid 40) is to a greater extent than the level of one or more other A β peptides, such as, *e.g.*, A β 40, is modulated. The level of A β 39 can be modulated without substantially altering the level of one or more other A β
20 peptides, such as, *e.g.*, A β 40. In a particular embodiment of these methods, the level of A β 39 is increased; in other embodiments, level of A β 39 is reduced. In particular embodiments of any of these methods, the level of the particular A β peptide, such as A β 42 or A β 39, can be changed by greater than or equal to about 50%. In one embodiment, A β 42 levels of the sample are reduced by greater than or equal to about
25 50%.

 In another embodiment of the methods for modulating A β levels, the cleavage of APP that produces one or more A β peptides, the processing of APP, the processing of A β and/or the levels of A β such that the level of A β 42 and the level of A β 39 are modulated to a greater extent than the level of one or more other A β peptides, such as, for example,
30 A β 40. In a further embodiment, the level of A β 42 and the level of A β 39 are modulated

- 187 -

without substantially altering the level of one or more other A β peptides, such as, for example, A β 40. In a particular embodiment of these methods, the level of A β 42 is reduced and the level of A β 39 is increased.

In particular embodiments of these methods, the sample contains APP and/or
5 portion(s) thereof. Samples that can be used include, but are not limited to, a cell, tissue, organism, cell or tissue lysate, cell or tissue extract, body fluid, cell membrane or composition containing cell membranes, and a cell-free extract or other cell-free sample.

In a particular embodiment, the sample contains a cell, including, for example, a eukaryotic cell such as a mammalian cell. Particular examples of mammalian cells
10 include rodent or human cells. In particular embodiments, the A β is cellular and/or extracellular A β .

In other embodiments of the methods for modulating A β levels, the method includes a step of modulating the cleavage of APP that produces one or more A β peptides, the processing of APP, the processing of A β and/or the level of one or more A β
15 peptides without substantially altering (a) one or more presenilin-dependent activities other than the presenilin-dependent processing of APP, (b) the cleavage and/or processing of a presenilin substrate and/or portion(s) thereof that is other than APP and/or (c) the cleavage and/or processing of LRP and/or portion(s) thereof. In particular embodiments of these methods, the levels of A β 42 are modulated. For example, the
20 levels of A β 42 may be modulated to a greater extent than the levels of other A β peptides, such as, for example, A β 40. The levels of A β 42 may be modulated without substantially altering the level of one or more other A β peptides, such as, *e.g.*, A β 40. In any of these embodiments, the level of A β 42 can be reduced or increased. In particular embodiments of these methods, the levels of A β 39 are modulated. For example, the levels of A β 39
25 may be modulated to a greater extent than the levels of other A β peptides, such as, for example, A β 40. The levels of A β 39 may be modulated without substantially altering the level of one or more other A β peptides, such as, *e.g.*, A β 40. In any of these embodiments, the level of A β 39 can be reduced or increased. In further embodiments, the levels of A β 42 and A β 39 are modulated. For example, the levels of A β 42 and A β 39 can be
30 modulated to a greater extent than the levels of other A β peptides, such as, *e.g.*, A β 40.

- 188 -

The levels of A β 42 and A β 39 levels can be modulated without substantially altering the level of one or more other A β peptides, such as, *e.g.*, A β 40. In particular embodiments, the level of A β 42 is reduced and/or the level of A β 39 is increased. In other
5 embodiments, the level of A β 42 is increased. In other embodiments, the level of A β 39 is decreased. In particular embodiments of any of these methods, the level of the particular A β peptide, such as A β 42 or A β 39, can be changed by greater than or equal to about 50%. In one embodiment, A β 42 levels of the sample are reduced by greater than or equal to about 50%.

10 The sample used in these methods can be any sample, such as those described herein. For example, the sample can contain a cell, a tissue, an organism, a cell or tissue lysate, a cell or tissue extract, a body fluid, a cell membrane or composition containing cell membranes, and/or a cell-free extract or other cell-free sample. The sample can contain presenilin (and/or portion(s) thereof), APP (and/or portion(s) thereof), and/or one or more presenilin substrates (and/or portion(s) thereof). In particular embodiments, the
15 sample contains one or more of: LRP, Notch, E-cadherin, TrkB, APLP2, hIre1 α , Erb-B4, portion(s) of LRP, portion(s) of Notch, portion(s) of E-cadherin, portion(s) of TrkB, portion(s) of APLP2, portion(s) of hIre1 α , and portion(s) of Erb-B4. In particular embodiments, the sample contains a cell, such as, for example, a eukaryotic cell, including, for example, a mammalian cell. Particular examples of mammalian cells
20 include rodent and human cells. In any of the methods, the A β can be cellular and/or extracellular A β .

 In particular embodiments of the methods for modulating A β levels that include a step of modulating the cleavage of APP that produces one or more A β peptides, the processing of APP, the processing of A β and/or the level of one or more A β peptides
25 without substantially altering the cleavage and/or processing of a presenilin substrate and/or portion(s) thereof that is other than APP, the presenilin substrate and/or portion(s) thereof, can be one or more of the following: Notch, E-cadherin, Erb-B4, and portions of Notch, E-cadherin and Erb-B4. In such embodiments, the modulation can be such that the levels of an intracellular carboxyl domain fragment of Notch, E-cadherin and/or Erb-
30 B4 are substantially unchanged.

- 189 -

In particular embodiments of the methods for modulating A β levels that include a step of modulating the cleavage of APP that produces one or more A β peptides, the processing of APP, the processing of A β and/or the level of one or more A β peptides without substantially altering the cleavage and/or processing of LRP and/or portion(s) thereof, the modulation can be such that the level and/or presence or absence of one or more fragments of LRP (and/or a portion(s) thereof) is substantially unchanged. In one embodiment the presence, absence and/or level of an ~20 kD fragment of LRP is substantially unchanged. The fragment can be one that (a) contains an amino acid sequence that is contained within a transmembrane region of LRP, (b) binds with an antibody generated against a C-terminal amino acid sequence of an LRP (*e.g.*, the C-terminal 13 amino acids of an LRP), (c) contains an amino acid sequence located within the sequence of amino acids from about amino acid 4420 to about amino acid 4544 of SEQ ID NO:10, (d) is present when an LRP is not cleaved by a presenilin-dependent activity, and/or (e) occurs in the presence of an inhibitor (*e.g.*, DAPT) of a presenilin-dependent activity. In one embodiment, the modulation can be such that the level and/or presence or absence of one or more C-terminal fragments (CTF) of LRP (and/or a portion(s) thereof) is substantially unchanged.

In any of the methods for modulating A β levels provided herein, the modulating can be effected by any method, including, but not limited to, contacting a sample with an agent that modulates the cleavage of APP that produces one or more A β peptides, the processing of APP, the processing of A β and/or the levels of A β such that the level of one or more A β peptides, such as, for example, A β 42, is modulated as described herein. An agent may be, for example, any agent identified using the methods provided herein for identifying agents that modulate A β . Agents include those that modulate the level, functioning and/or activity of one or more proteins involved in modulating A β . Proteins involved in modulating A β can be, for example, APP processing enzymes, A β processing enzyme, receptors or modulatory proteins thereof. In particular examples, the concentration of the agent is less than or equal to about 35 μ M, 30 μ M, 25 μ M, 20 μ M, 15 μ M or 10 μ M. For example, the concentration of agent is less than or equal to about 30 μ M. In one embodiment, the agent reduces A β 42 levels with an IC₅₀ of about 25 μ M

- 190 -

or less or about 20 μ M or less.

I. Antibodies and Proteins that bind A β

Provided herein are antibodies and methods of preparing antibodies which are specifically reactive with A β . Also provided are proteins engineered to bind A β . Such antibodies and A β binding proteins can be used in applications such as, but not limited to, diagnostic purposes, research purposes, and in treatment of A β -related diseases and conditions. For example, A β binding proteins can be used as reagents for the assays and kits described herein for the detection of the modulation or processing of APP.

Antibodies and antibody fragments described herein for use in immunological detection of A β , such as those used in assays to monitor APP processing and modulation, can also be used in other applications such as diagnostic purposes, research purposes, and in treatment of A β -related diseases and conditions. A β binding proteins including A β antibodies can also be used as candidate agents as described herein for modulating A β levels.

1. A β Antibodies

A β antibodies provided herein are specifically reactive with A β . In one embodiment, antibodies which are specifically reactive with A β recognize the N-terminal region of A β . Antibodies which recognize the N-terminal region of A β can be prepared by immunizing a host animal with a peptide containing the sequence of the N-terminal region of A β . For example, a peptide containing the sequence of amino acids 1-12 of SEQ ID NO: 4 or a fragment thereof is used to immunize mice and generate monoclonal antibodies as described herein or by method known in the art. An exemplary antibody is the A β antibody A β 1-12, referred to herein as B436.

In another embodiment, antibodies are prepared which recognize only a particular A β or a selective number of A β peptides. Antibodies can be prepared by immunizing a host animal such as a mouse with portions of A β specific for the species of interest. For example, as described herein, antibodies can be generated which recognize only A β 42 with minimal or no binding to other A β peptides, such as A β 40. An A β antibody selective for A β 42 can have at least about 100-fold, 200-fold, 300-fold, 400-fold, 500-fold, 600-fold, 700-fold, 800-fold, 900-fold, 1000-fold or more specificity or selectivity

- 191 -

for A β 42 relative to other forms of A β , such as A β 40. In addition, the antibody can have an affinity constant for binding to A β 42 of at least about 10^5 l/mol, 2×10^5 l/mol, 3×10^5 l/mol, 4×10^5 l/mol, 5×10^5 l/mol, 6×10^5 l/mol, 7×10^5 l/mol, 8×10^5 l/mol, 9×10^5 l/mol, 10^6 l/mol, 2×10^6 l/mol, 3×10^6 l/mol or 4×10^6 l/mol or more. An exemplary antibody is the A β antibody selective for A β 42, referred to herein as A387.

A β antibodies can be produced which recognize some or all forms of A β , for example A β in soluble form, such as in low molecular weight forms, in plaques and in neurofibrillary tangles. A β antibodies can also be produced which recognize only a specific A β , for example A β 42, and in some cases are also specifically reactive with specific forms of A β , for example A β 42 in soluble form such as A β 42 in plasma and A β 42 in low molecular weight forms. A β antibodies which recognize only specific A β peptides, and/or are specifically reactive with specific forms of A β can be used to ascertain the form(s) and types of A β peptides in a sample, for purposes of diagnosis, such as in methods described herein or known in the art. Such A β antibodies can be used for treatment for example, where a predominant form and/or A β peptide is associated with an A β -related condition or the modulation of a form and/or a particular A β is effective for treatment.

Antibodies can be prepared using a variety of methods well-known in the art. For example, as described herein, a target epitope such a peptide, peptide fragment or synthetic peptide may be prepared and used to immunize a host animal. As further described herein, monoclonal antibodies can be prepared, cell lines producing monoclonal antibodies can be isolated and the nucleic acid sequence encoding the monoclonal antibodies as well as the amino acid sequence of the antibodies can be obtained.

An antibody can be any derivative of an immunoglobulin. A β antibodies include antibodies that are less than full-length, *e.g.* antibody fragments, retaining at least a portion of the full-length antibody's specific binding ability. Examples of such antibodies include, but are not limited to, Fab, Fab', F(ab)₂, single-chain Fvs (scFv), Fv, dsFv and diabody fragments. Antibodies can include multiple chains linked together, such as by disulfide bridges. Antibodies can be prepared enzymatically and by

- 192 -

recombinant DNA technology.

(a) Fab and F(ab)₂ fragments

Fab fragments are antibody fragments that can be produced from digestion of an immunoglobulin with papain. A Fab fragment contains a complete light chain paired
5 with the variable region and the C_H1 region of the heavy chain. Recombinant means such as expression in a host cell, synthetic production or *in vitro* expression systems can also be used to produce Fab fragments of similar or equivalent structure to Fab fragments produced by enzymatic digestion .

10 Fab fragments can be generated which are specifically reactive with A β or with particular A β peptides. In one embodiment, an Fab recognizes all or most A β peptides. For example, an Fab is produced which recognizes the N-terminal amino acids of A β such as an Fab generated from the antibody B436 or an Fab produced using the sequence or a portion of the sequence of the B436 antibody.. In another embodiment, an Fab is specifically reactive with a specific A β , for example, A β 42.

15 Fab fragments can be produced by enzymatic means. For example, an Fab can be generated from A β antibodies such as A387 and/or B436 by isolating immunoglobulin from antibody producing cells, such as described in the examples herein or by methods known in the art. Fab antibodies are generated by cleaving the A387 and/or B436 immunoglobulin molecules with papain.

20 In another embodiment, Fab molecules are generated from A β antibodies such as A387 and/or B436 by recombinant means using the sequences of the light and heavy chain variable regions and mimicking the papain cleavage by constructing the polypeptides of the heavy and light chain variable domains to have the same or similar (within 1 or more amino acids in length difference) amino acid sequences. For example,
25 A387 Fab molecules can be constructed containing the amino acid sequences or a portion thereof, of SEQ ID NOs: 12 and 14. In one aspect of the embodiment, nucleic acid molecules containing the sequence or a portion thereof of SEQ ID NO:11 and/or 13 are used to construct an Fab antibody. B436 Fab molecules can be constructed containing the amino acid sequences or a portion thereof, of SEQ ID NOs: 16 and 18. In one aspect
30 of the embodiment, nucleic acid molecules containing the sequence or a portion thereof

- 193 -

of SEQ ID NO:15 and/or 17 are used to construct an Fab antibody.

An F(ab)₂ fragment is an antibody fragment that can be produced from digestion of an immunoglobulin with pepsin at pH 4.0-4.5. An F(ab)₂ fragment contains both light chains associated with the variable regions and the C_H1 regions of the two heavy chains.

- 5 Disulfide bridges link the two antigen binding arms of the F(ab)₂ fragment. Recombinant means such as expression in a host cell, synthetic production or *in vitro* expression systems can also be used to produce F(ab)₂ fragments of similar or equivalent structure to F(ab)₂ fragments produced by enzymatic digestion.

- 10 F(ab)₂ fragments can be produced which are specifically reactive with A β and/or specific A β peptides. In one embodiment, an F(ab)₂ fragment recognizes the N-terminal amino acids of A β such as an F(ab)₂ from the antibody B436 or an F(ab)₂ produced using the sequence or a portion of the sequence of the B436 antibody. In another embodiment, an F(ab)₂ is specifically reactive with a specific A β , for example, A β 42. For example, an F(ab)₂ is generated from the antibody A387 or an F(ab)₂ is produced using the sequence
15 or a portion of the sequence of the A387 antibody.

- F(ab)₂ fragments can be produced by enzymatic means. For example, an F(ab)₂ can be generated from A β antibodies such as A387 and/or B436 by isolating immunoglobulin from antibody producing cells, such as described in the examples herein or by methods known in the art. F(ab)₂ antibodies are generated by cleaving the A387
20 and/or B436 immunoglobulin molecules with pepsin.

- In another embodiment, F(ab)₂ molecules are generated from A β antibodies such as A387 and/or B436 by recombinant means using the sequences of the light and heavy chain variable regions and mimicking the pepsin cleavage by constructing the polypeptides of the heavy and light chains to have the same or similar (within 1 or more
25 amino acids in length difference) amino acid sequences. For example, A387 F(ab)₂ molecules can be constructed containing the amino acid sequences or a portion thereof, of SEQ ID NOs: 12 and 14. In one aspect of the embodiment, nucleic acid molecules containing the sequence or a portion thereof of SEQ ID NO:11 and/or 13 are used to construct an F(ab)₂ antibody. B436 F(ab)₂ molecules can be constructed containing the
30 amino acid sequences or a portion thereof, of SEQ ID NOs: 16 and 18. In one aspect of

- 194 -

the embodiment, nucleic acid molecules containing the sequence or a portion thereof of SEQ ID NO:15 and/or 17 are used to construct an F(ab)₂ antibody.

(b) Fv and dsFv fragments

An Fv antibody fragment is composed of one variable heavy domain (V_H) and
5 one variable light domain linked by noncovalent interactions. Fv fragments can be generated by recombinant DNA technology produce the variable domains of the heavy and light chains, for example in a host cell, or by synthetic means. In one embodiment, an Fv fragment is generated from the A387 by recombinant means using nucleotide sequences encoding the heavy chain and light chain variable domains set forth in SEQ ID
10 NO:12 and 14. In one aspect of the embodiment, nucleic acid molecules containing the sequence or a portion thereof of SEQ ID NO:11 and/or 13 are used to construct an Fv fragment.

In another embodiment, Fv fragments are generated by recombinant means using nucleotide sequences encoding the heavy chain and light chain variable domains set forth
15 in SEQ ID NO:16 and 18. In one aspect of the embodiment, nucleic acid molecules containing the sequence or a portion thereof of SEQ ID NO:15 and/or 17 are used to construct an Fv fragment.

A dsFV refers to an Fv with an engineered intermolecular disulfide bond, which stabilizes the V_H-V_L pair. Chain dissociation may be prevented by introducing Cys
20 residues at appropriate locations into the framework of V_H and V_L in order to form a disulphide crosslink (Glockshuber *et al.*, 1990; Reiter *et al.*, 1996). dsFv molecules can be generated by recombinant means to produce dsFv antibodies from A387 and/or B436. For example, cysteines can be engineered into the sequence of the heavy and light chains to provide a disulfide bond between them. dsFvs can then be generated by enzymatic or
25 by recombinant means.

(c) ScFvs and diabodies

scFvs refer to antibody fragments that contain a variable light chain (V_L) and variable heavy chain (V_H) covalently connected by a polypeptide linker in any order. The linker is of a length such that the two variable domains are bridged without substantial
30 interference. Included linkers are (Gly-Ser)_n residues with some Glu or Lys residues

- 195 -

dispersed throughout to increase solubility. scFvs are generated by recombinant means and may be produced synthetically, *in vivo*, such as by expression in a host cell or transgenic organism, or using *in vitro* systems known in the art. scFvs can be advantageous because of the smaller size.

5 scFvs can be generated which are specifically reactive with to A β or to specific A β peptides. In one embodiment, an scFv is produced which recognizes the N-terminal region of A β . For example, an scFv is generated using the sequence of the antibody B436 or a portion thereof, such as the sequence comprising the variable regions of the heavy and light chain of B436. A linker region is used such as those described herein or
10 known in the art to join the variable regions. In another embodiment, an scFv is generated which recognizes specific A β peptide, for example, an scFv which are specifically reactive with A β 42. In one embodiment, an scFv is generated using the sequence of the antibody A387 or a portion thereof, such as the sequence comprising the variable regions of the heavy and light chain of A387. A linker region is used such as
15 those described herein or known in the art to join the variable regions. For example, an scFv is generated containing the sequence of amino acids or a portion thereof of SEQ ID NO:12 and/or 14. In another embodiment, an scFv is generated using the sequence of the antibody B436 or a portion thereof, such as the sequence comprising the variable regions of the heavy and light chain of B436. A linker region is used such as those described
20 herein or known in the art to join the variable regions. For example, an scFv is generated containing the sequence of amino acids or a portion thereof of SEQ ID NO:16 and/or 18.

(d) Complementarity-determining Regions (CDRs)

Complementarity-determining regions (CDRs) (also referred to as hypervariable regions) refer to regions of an immunoglobulin molecule that vary greatly in amino acid
25 sequence relative to flanking Ig sequences. The length and conformation of CDRs vary among Igs, but generally CDRs form short loops supported by a sandwich of two anti-parallel beta-sheets within the variable regions of the antibody. Three CDRs, designated CDR-L1, CDR-L2 and CDR-L3, are present in the variable region of an immunoglobulin light chain, and three CDRs, designated CDR-H1, CDR-H2 and CDR-H3, are present in
30 the variable region of an immunoglobulin heavy chain. Each CDR generally contains at

- 196 -

least one, and often several, amino acids residues that make contact with antigen, but all six CDRs are not necessarily required to maintain the binding specificity of an antibody.

Several definitions of CDRs are commonly in use, and CDRs identified according to the different definitions generally overlap, but may differ slightly in their boundaries.

- 5 The Kabat CDR definition is based on sequence variability among immunoglobulins. The Chothia CDR definition is based on the location of structural loop regions. The AbM CDR definition is a compromise between the Kabat and Chothia definitions used by Oxford Molecular's AbM antibody modeling software. The contact CDR definition is based on a comparison of the available complex crystal structures.
- 10 Taking into account these alternative CDR definitions, some general principles have been devised to identify CDRs based on a given amino acid sequence are shown in Table 3 (see, for example, www.bioinf.org.uk/abs/).

Table 3

	Start	Residue(s) before	Residue(s) after	Length	Alternatives
CDR-L1	~ residue 24	Usually C	Usually W	~10-17 residues	
CDR-L2	Usually 16 residues after end of L1	Usually I- Y, V-Y, I- K or I-F		Usually 7 residues	
CDR-L3	Usually 33 residues after end of L2	Usually C	Usually F- G-X-G	~7-11 residues	
CDR-H1	~ residue 26	Usually C- X-X-X	Usually W	~10-12 residues	Kabat definition starts 5 residues later (length is ~5-7 residues)

- 197 -

					Chothia definition ends 4 residues earlier (length is ~6-8 residues)
CDR-H2	Usually 15 residues after end of H1	Often similar to L-E-W-I-G	Often K/R/L-L/I/V/F/A-T/S/I/A	~16-19 residues	AbM and Chothia definitions end 7 residues earlier (length is ~9-12 residues)
CDR-H3	Usually 33 residues after end of H2	Usually C-X-X	Usually W-G-X-G	~3-25 residues	

Applying these principles to the antibody sequences disclosed herein, exemplary CDR sequences of the A387 and B436 antibodies can be defined as shown in Table 4.

5 Table 4

	Exemplary CDR sequence	Exemplary CDR sequences according to alternative CDR definition(s)
A387 CDR-L1	RASQSI>NNLH (aa 24-34 of SEQ ID NO:12)	
A387 CDR-L2	YASQSIY (aa 50-56 of SEQ ID NO:12)	
A387 CDR-L3	QQSHSWPLT (aa 89-97 of SEQ ID NO:12)	
A387 CDR-H1	GFTFSNDAMS (aa 26-35 of SEQ ID NO:14)	NDAMS (Kabat) (aa 31-35 of SEQ ID NO:14) GFTFSN (Chothia)

- 198 -

		(aa 26-31 of SEQ ID NO:14)
A387 CDR-H2	SISSVGNTYYPDSVKG (aa 50-65 of SEQ ID NO:14)	SISSVGNTY (Chothia and AbM) (aa 50-58 of SEQ ID NO:14)
A387 CDR-H3	GYGVSPWFSY (aa 98-107 of SEQ ID NO:14)	
B436 CDR-L1	RSSQNIVHSSGNTYLE (aa 24-39 of SEQ ID NO:16)	
B436 CDR-L2	KVSNRFS (aa 55-61 of SEQ ID NO:16)	
B436 CDR-L3	FQGSHVPYT (aa 94-102 of SEQ ID NO:16)	
B436 CDR-H1	GFTFSRYTMS (aa 26-35 of SEQ ID NO:18)	RYTMS (Kabat) (aa 31-35 of SEQ ID NO:18) GFTFSR (Chothia) (aa 26-31 of SEQ ID NO:18)
B436 CDR-H2	TINFGNGNTYYPDSVKG (aa 50-66 of SEQ ID NO:18)	TINFGNGNTY(Chothia and AbM) (aa 50-59 of SEQ ID NO:18)
B436 CDR-H3	LNWAY (aa 99-103 of SEQ ID NO:18)	

Thus, as used herein, a "CDR of antibody A387" refers to a sequence of amino acids that is a) the same as one of the amino acid sequences set forth in rows 2-7 of Table 4; b) a fragment of SEQ ID NO:12 or 14 with N- and/or C-terminal boundaries that differ by no more than about 4, 3, 2 or 1 amino acids relative thereto; or c) is at least 60%, 65%, 70%, 80%, 85%, 90%, 95% or more identical to a) or b). A CDR of antibody A387 also includes substitutions within the amino acid sequences of the CDRs set forth in rows 2-7 of Table 4 that when substituted into an A387 antibody do not substantially alter the binding affinity or selectivity of the antibody as compared with the unmodified A387

- 199 -

antibody. Such substitutions can be conservative amino acid substitutions (for example, conservative amino acid changes set forth in Table 2). Generally such substitutions can be for example, 1 amino acid change or 2 amino acid changes within a CDR sequence set forth in rows 2-7 of Table 4.

5 As used herein, a "CDR of antibody B436" refers to a sequence of amino acids that is a) the same as one of the amino acid sequences set forth in rows 8-13 of Table 4 b) a fragment of SEQ ID NO:16 or 18 with N- and/or C-terminal boundaries that differ by no more than about 4, 3, 2 or 1 amino acids relative thereto; or c) is at least 60%, 65%, 70%, 80%, 85%, 90%, 95% or more identical to a) or b). A CDR of antibody B436 also
10 includes substitutions within the amino acid sequences of the CDRs set forth in rows 8-13 of Table 4 that when substituted into a B436 antibody do not substantially alter the binding affinity or selectivity of the antibody as compared with the unmodified B436 antibody. Such substitutions can be conservative amino acid substitutions (for example, conservative amino acid changes set forth in Table 2). Generally such substitutions can
15 be for example, 1 amino acid change or 2 amino acid changes within a CDR sequence set forth in rows 8-13 of Table 4.

One or more, up to all of the CDRs of an A β antibody can be used to bind A β or a specific form of A β . The CDRs may be produced by recombinant means such as produced synthetically, *in vivo*, such as by expression in a host cell or transgenic
20 organism, or using *in vitro* systems known in the art. CDRs may be produced as isolated sequences or may comprise a portion of a larger molecule such as an immunoglobulin, an Fab, F(ab)₂, an scFv, diabody or a chimeric polypeptide. Multimerization of antibody fragments or antibody domains can be used increase the avidity of such molecules for A β and/or specific A β peptides and/or forms of A β . Chemical means, such as by
25 crosslinking or disulfide bond formation can be used to generate multimeric forms of antibodies. Recombinant means can also be used, for example by constructing repetitive domains or by introducing functionalities which can then be used for cross-linking or association by other means.

2. Engineering A β binding proteins

30 Antibodies or regions thereof, such as CDRs, can be engineered to generate A β

- 200 -

binding proteins which bind A β or particular peptides or forms of A β . For example A β binding proteins can be engineered to optimize the binding to A β and/or a particular A β and/or specific forms of A β , to optimize attributes for specific uses such as treatment or diagnostic methods, optimize attributes for production or other desirable characteristics.

5 In one embodiment, an A β binding protein is generated which binds to a particular A β and/or binds selectively to one or more A β peptides. For example, an A β binding protein is engineered to retain substantially the same binding properties as an A β antibody. In one embodiment, an A β binding protein is engineered to retain substantially the same binding properties as the A387 antibody. In another embodiment, an A β
10 binding protein is engineered to retain substantially the same binding properties as the B436 antibody.

As described herein, A β binding proteins can be generated which recognize only A β 42 with minimal or no binding to other A β peptides, such as A β 40. An A β binding protein selective for A β 42 can have at least about 100-fold, 200-fold, 300-fold, 400-fold,
15 500-fold, 600-fold, 700-fold, 800-fold, 900-fold, 1000-fold or more specificity or selectivity for A β 42 relative to other forms of A β , such as A β 40. In addition, the A β binding protein can have an affinity constant for binding to A β 42 of at least about 10^5 l/mol, 2×10^5 l/mol, 3×10^5 l/mol, 4×10^5 l/mol, 5×10^5 l/mol, 6×10^5 l/mol, 7×10^5 l/mol, 8×10^5 l/mol, 9×10^5 l/mol, 10^6 l/mol, 2×10^6 l/mol, 3×10^6 l/mol or 4×10^6 l/mol
20 or more.

A β binding proteins can be generated for example, from portions of antibodies that recognize A β can be engineered into other protein scaffolds. Nucleic acid molecules encoding such portions along with nucleic acid molecules encoding scaffolds can be used to construct A β binding proteins including A β antibodies using standard molecular
25 biology techniques known to one skilled in the art. Exemplary nucleic acid molecules include but are not limited to SEQ ID NOs. 11, 13, 15, 17, 97, 98, 99 and 100. Additionally, nucleic acid molecules can be generated by reverse translating A β binding protein amino acid sequences. For example, a nucleic acid sequence is derived from a portion of an A β antibody, such as a CDR amino acid sequence. There are a number of
30 possible nucleic acid sequences based on the degeneracy of codons which can be used for

- 201 -

each amino acid. However, for the purposes of constructing A β binding proteins, any nucleic acid sequence which encodes the amino acid sequence can be used for constructing an A β binding protein. Nucleic acid molecules encoding A β binding proteins, antibodies or portions thereof can be mutagenized to alter binding characteristics. Additional functionalities such as detectable moiety or a therapeutic moiety can be added to A β binding proteins and antibodies. Protein and peptide chemistry can also be used to construct A β binding proteins.

(a) Scaffolds

A scaffold refers to a structure that forms a conformationally stable structural support, or framework, which is able to display one or more sequences of amino acids, such as a CDR, a variable region or a binding domain, in a localized surface region. A scaffold may be a naturally occurring polypeptide or polypeptide "fold" (a structural motif), or may have one or more modifications, such as additions, deletions or substitutions of amino acids, relative to a naturally-occurring polypeptide or fold. A review of protein scaffolds and their uses can be found in Skerra (2000) *J. Mol. Recognition* 13:167-187.

i. Antibody Scaffolds

Immunoglobulins comprise a natural type of biomolecular scaffold. A β binding proteins can be engineered based on immunoglobulin molecules or portions thereof including, CDR grafting, humanized antibodies, single Ig and Ig-like scaffolds and antibody fragments such as Fvs, scFvs, Fabs, and F(ab)₂s.

Accordingly, provided herein are antibodies and antibody fragments for use as antibody scaffolds. Such scaffolds can contain the heavy and/or light chains of an immunoglobulin or portions thereof. In one embodiment, an antibody scaffold is constructed from a heavy chain. The heavy chain can be from an A β antibody such as from A387 or B436 or from any heavy chain known in the art. In another embodiment, an antibody scaffold is constructed from the constant region of one antibody and the variable region from an A β antibody. For example, the C region can contain the sequence of amino acids set forth in SEQ ID NOs: 69, 71, 83, 85 or 87 and the variable

- 202 -

region can contain the amino acids of SEQ ID NO 14 or 18 or a portion thereof. A joining region can be used from either an A β antibody or from an antibody known in the art. Exemplary joining regions are described herein. In a particular embodiment, an antibody scaffold contains a variable region containing the sequence of amino acids 1-97
5 of SEQ ID NO:14 or 1-98 of SEQ ID NO: 18. In another embodiment, an antibody scaffold is constructed from a light chain. The light chain may be from an A β antibody or from any light chain known in the art. In another embodiment, an antibody scaffold is constructed from the constant region of one light chain and the variable region from an A β antibody. For example, the C region can contain the sequence of amino acids set
10 forth in SEQ ID NOs: 63, 65 or 81 and the variable region can contain the amino acids of SEQ ID NO 12 or 16, or a portion thereof. A joining region can be used from either an A β antibody or from an antibody known in the art. Exemplary joining regions are described herein. In a particular embodiment, an antibody scaffold contains a variable region containing the sequence of amino acids 1-95 of SEQ ID NO:14 or 1-100 of SEQ
15 ID NO:16. Heavy and light chains can also be constructed containing a portion of an antibody known in the art and a portion of an A β antibody, for example by grafting the variable domain of an A β heavy chain, the DJ region and a portion of the C domain to another heavy chain containing the remainder of the C domain, thereby reconstructing a heavy chain. In another example, a light chain can be constructed by the variable domain
20 of an A β light chain, the J region and a portion of the C domain to another light chain containing the remainder of the C domain, thereby reconstructing a light chain

Antibody scaffolds can be constructed for Fab, F(ab)₂, Fvs, dsFvs, diabodies and other antibodies by methods as described herein or known in the art. Scaffolds for antibodies can also be constructed by utilizing other antibodies known in the art and
25 altering the binding specificity such that antibody recognizes A β . For example, the variable region or a portion thereof can be grafted onto the antibody or used to replace the equivalent region within the scaffold. Single CDR regions can be grafted and/or used for replacement as well as all of the CDR regions of the light chain and/or heavy chain or any combination thereof. Mutagenesis can also be used to alter the binding specificity of
30 an existing antibody such that it binds A β .

- 203 -

Antibody scaffolds can also be used to generate antibodies with the specificity from one antibody and the properties of another, such as reduced immunogenicity when administered in a particular animal species. Monoclonal antibodies are most often generated in non-human species, such as mice. Humanized antibodies can be generated where at least one portion of the antibody structure is of human origin. For example, a humanized antibody can be comprised of the antigen binding regions from an antibody generated in a mouse with the remainder of the antibody framework derived from a human antibody (see, for example, Hurlle and Gross, Curr Opin Biotechnol. 1994 Aug;5(4):428-33). The generation of humanized antibodies includes the methods referred to in the art as CDR-grafting. Humanized antibodies can be prepared by synthetic methods or through recombinant DNA methods well known in the art.

Accordingly, provided herein are humanized antibodies which bind to A β . In one embodiment, one or more CDRs of an A β antibody is grafted onto a human antibody framework such as an Fab and scFv framework. For example, one or more of the CDRs of the A β antibody A387 is grafted onto a human antibody framework to create a humanized A β antibody. A387 CDRs can be any one or more than one of the CDRs listed in Table 4 for A387 including A387 CDR L1, L2, L3, H1, H2 and H3 in any combination. A387 CDRs also include fragments of the amino acid sequences set forth in SEQ ID NO:12 and SEQ ID NO:14. In another embodiment, one or more of the CDRs of the A β antibody B436 is grafted onto a human antibody framework. B436 CDRs can be any one or more than one of the CDRs listed in Table 4 for B436 including B436 CDR L1, L2, L3, H1, H2 and H3 in any combination. B436 CDRs also include fragments of the amino acid sequences set forth in SEQ ID NO:16 and SEQ ID NO:18. In one embodiment, the humanized antibodies contain the 6 CDRs of an A β antibody, for example, a humanized antibody with the 6 CDRs of A387. In another example, a humanized antibody contains the 6 CDRs of antibody B436.

Any human antibody framework known in the art can be used to prepare humanized antibodies. For example, a human framework can be a human scFv antibody, a human Fab fragment, a human light chain, a human heavy chain or a full immunoglobulin structure comprised of both a heavy and a light chain. Exemplary

- 204 -

human immunoglobulin regions useful in constructing scaffolds are those such as, but not limited to, polypeptides set forth in SEQ ID NOs: 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, and 91.

Additionally, a human antibody framework may be optimized for example to
5 improve solubility properties or increase production in a host. For example, a camelized version of a human V_H domain can be constructed as a human antibody fragment or as a portion of a larger human antibody framework (see for example, Davies and Riechmann (1995) *Bio/technology* 13:475-479 and Davies and Riechmann (1996) *Prot. Eng* 9:531-537). CDR grafting can be used to engineer A β binding proteins in Ig chain scaffolds
10 such as single Ig and Ig-like scaffolds. For example, camelid antibodies are heavy chain antibodies which are devoid of light chains so that their V_H domains remain soluble without dimerization. An A β binding protein can be constructed, for example, by grafting one or more of the CDRs of an A β antibody into the camelid antibody structure. Human and murine variable domains have been described, which do not depend on the
15 association with another domain and can be used to create a single Ig-like scaffold for an A β binding protein. An additional small Ig-like framework is the minibody, for example, based on the heavy chain variable domain of an antibody comprising three strands from each β -sheet and having regions that structurally correspond to CDR-H1 and CDR-H2. Minibodies also generally contain a metal-binding site and solubilizing tri-lysine motifs
20 at the N- or C-termini (Bianchi *et al.* (1994) *J. Mol. Biol.* 236:649-659). Isolated V_H domains containing CDR1 and CDR2 and associated framework can also be used (Davies *et al.*, (1995) *Biotechnology* 13:475-479). CDR regions of an A β antibody such as the CDR-H1 and CDR-H2 regions from the A387 or B436 antibodies can be used to construct A β minibodies.

25 An example of a single Ig-like scaffold is the fibronectin type III domain (FN3) which constitutes a small, monomeric natural β -sandwich protein with resemblance to a trimmed Ig V_H domain. It possesses seven β -strands with three loops connecting the strands in a pairwise fashion at one end of the β -sheet. The loops can be replaced with one or more CDRs from an A β antibody to create an A β binding protein with a
30 fibronectin scaffold. FN3 domains are found in numerous binding proteins, such as cell

- 205 -

adhesion molecules, cell surface hormone and cytokine receptors, chaperonins and carbohydrate-binding proteins, and generally contain seven β -strands with three loops connecting the strands in a pairwise fashion at one end of the β -sheet. An exemplary FN3 domain scaffold is derived from the tenth FN3 repeat in human fibronectin (Koide
5 *et al.* (1998) *J. Mol. Biol.* 284:1141-1151; WO 98/56915; WO 02/04523). Another example of a single Ig-like domain scaffold is the V-like domain of the human cytotoxic T-lymphocyte associated protein-4 (CTLA-4) (Nuttall *et al.* (1999) *Proteins Struct. Funct. Genet.* 36:217-227).

ii. Other Polypeptide Scaffolds

10 Beyond antibody scaffolds, other proteins with suitable architecture can be used as scaffolds to create $A\beta$ binding proteins. Many of these proteins have defined folds and loops that are appropriate for insertion or replacement with $A\beta$ binding regions such as one or more CDRs of an $A\beta$ antibody. A scaffold may be derived from a polypeptide of any species (or of more than one species), such as a human, other mammal, other
15 vertebrate, invertebrate, plant, bacteria or virus or may be generated by rational design (*e.g.* an artificial scaffold).

Protease inhibitors generally have a binding site that comprises an exposed loop in a context of a structural framework that is specific for the inhibitor family and thus can be employed as a scaffold for a structurally constrained peptide loop Roberts *et al.* (1992)
20 *Proc. Natl. Acad. Sci. USA* 89:2429-2433; Markland *et al.* (1996) *Biochemistry* 35:8045-8057; McConnell and Hoess (1995) *J. Mol. Biol.* 250:460-470). Protease inhibitor scaffold include but are not limited to scaffolds from Bovine (or basic) pancreatic trypsin inhibitor, BPTI, the Kunitz domain of human lipoprotein-associated coagulation inhibitor (LACI-D1), human pancreatic secretory trypsin inhibitor (PSTI), bacterial serine protease
25 inhibitor ecotin, and Tendamistat. The exposed loop may be replaced by one or more CDRs of an $A\beta$ antibody to create an $A\beta$ binding protein.

Helical bundle proteins can also be used as scaffolds (Braisted and Wells (1996) *Proc. Natl. Acad. Sci. USA* 93:5688-5692; Ku and Schultz (1995) *Proc. Natl. Acad. Sci. USA* 92:6552-6556). For example, an engineered single domain, called 'Z', of
30 *Staphylococcal* protein A has a simple fold as a bundle of three α -helices. It is highly

- 206 -

soluble and stable against proteolysis and heat-induced unfolding. Another example is cytochrome *b*₅₆₂, with four-helix bundle proteins providing rigid framework and two loops, each connecting one pair of the α -helices. Artificial helical bundle scaffolds are also available. One of more CDR regions from an A β antibody can be grafted into the
5 helical structure for example, into the loop regions between one or more of the helices to create an A β protein.

An additional scaffold is the β -barrel which is made of antiparallel β -strands winding around a central axis with loops connecting the strands at the open end of the resulting conical structure. For example, the β barrel framework of lipocalins (Muller
10 and Skerra (1994) *Biochemistry* 33:14126-14135) may be used such as by grafting of a domain onto the solvent-exposed outer surface of the β -barrel. One or more CDRs of an A β antibody can be grafted onto a lipocalin scaffold. Examples of lipocalin scaffold include but are not limited to retinol-binding protein (RBP), bilin binding protein (BBP), apolipoprotein D, tear lipocalin and β -Trace, also known as prostaglandin D synthase.
15 Many lipocalins based on their human framework and natural presence in human body fluids are suitable both for diagnostic and therapeutic purposes.

Knottins (Le Nguyen *et al.*, 1990) comprise a structural family defined by a small triple-stranded antiparallel β -sheet stabilized by an arrangement of disulphide bonds. Members of the knottin family include the trypsin inhibitor EETI-II from *Ecballium*
20 *elaterium* seeds, the neuronal N-type Ca²⁺ channel blocker (ω -conotoxin from the venom of the predatory cone snail *Conus geographus*, and the C-terminal cellulose-binding domain (CBD) of cellobiohydrolase I from the fungus *T. reesei*. Loop structures within the Knottins can be used for insertion of or replaced with one or more CDR sequences to form A β binding proteins.

25 Other structural folds that may be suitable as scaffolds include TIM barrels, which are found, for example, in triose phosphate isomerase proteins (Altamirano *et al.* (2000) *Nature* 403:617-622); GST enzyme frameworks, pleckstrin homology domains, zinc finger domains and β -prism motifs.

Exemplary modifications to a polypeptide that may make it suitable for use as a
30 scaffold include deletions of those regions that form binding loops in the naturally-

- 207 -

occurring molecule (*e.g.* deletions of the naturally-occurring binding sites); deletions of those regions that are unnecessary for structural integrity of the fold; substitutions of amino acids that flank the loop regions with residues that improve the properties of the polypeptide (such as improved affinity, specificity, or solubility; reduced immunogenicity, etc.); addition of detectable sequences, such as epitope tags; and the like.

iii. Non-polypeptide Scaffolds

A β antibodies and A β binding proteins, fragments thereof, such as a CDR, can also be displayed on a scaffold such as a solid support. Such scaffolds are useful in applications including but not limited to, diagnostic assays, screening assays, and cellular delivery of polypeptides.

Solid supports include but are not limited to membranes, filters, chips, slides, wafers, fibers, magnetic or nonmagnetic beads, gels, tubing, plates, polymers, microparticles and capillaries. A solid support can be composed of any material that allows for the immobilization or attachment of molecules, such that these molecules retain their desired properties, such as binding ability. Examples of materials include silica, polymeric materials or glass. Solid supports can be used to display A β binding proteins, antibodies and fragments thereof, for example for screening purposes, diagnostic purposes, protein purification and binding assays. Additionally, solid supports such as beads and particles can be used to deliver A β binding proteins and antibodies to cells, animals and subjects. A β binding proteins, antibodies and fragments thereof can be associated with solid supports covalently such as by chemical linkage or by non-covalent interactions such as by charge interactions, interactions with other proteins or small molecules.

(b) Mutagenesis of A β binding regions

As described herein, A β binding proteins can be constructed from A β binding regions such as A β antibodies and antibody fragments including one or more CDRs. Properties of such A β binding proteins can be altered or optimized. For example properties such as binding affinity, binding specificity, solubility, aggregation and stability can be optimized for particular applications.

- 208 -

Mutagenesis techniques such as site-directed mutagenesis, random mutagenesis including random mutagenesis of discrete regions of A β binding proteins and other methods known in the art can be used to generate variations within the A β binding regions, or at one or more junctions between the A β binding regions and the scaffold.

- 5 The variants can then be screened for A β binding by methods such as described herein or known in the art and variants with improved binding affinities or binding affinities optimized for particular applications such as diagnostics or treatment regimes can be isolated. For example, one or more CDRs of an A β antibody such as the CDRs of A387 and/or B436 can be mutagenized and then the variants generated are tested for A β
- 10 binding. Random mutagenesis or directed conservative amino acid changes can be made in one or more CDRs. The variants can also be tested for selective binding to one or more specific A β peptides such as binding to A β 42, or A β 1-12. The variants can be screened to assess for their binding to specific forms of A β . For example, variants can be assayed for their binding to A β in plasma, cerebral spinal fluid (CSF), plaques, and
- 15 neurofibrillary tangles as well as in low molecular weight and high molecular weight forms.

- 20 Variants can also be assessed for properties other than binding to A β . For example, variants can be isolated which are more soluble when produced synthetically or in a host by recombinant means. Variants can also be isolated which exhibit altered stability, for example increased stability or alternatively higher turnover. Such variants can be produced by mutagenizing regions outside the A β binding regions for example in the scaffold, antibody framework or other domains which are part of the A β binding protein. Such variants can also be produced by mutagenizing the A β binding regions or the entire A β binding protein and then screened for retention of A β binding as one of the
- 25 criteria for selecting a variant.

(c) Clearance domains

- A clearance domain directly or indirectly mediates enhanced clearance of a polypeptide from the circulation. A polypeptide containing a clearance domain will have a shorter half-life in the circulation, alone and/or when bound to A β , than a polypeptide
- 30 without such a domain. Clearance mechanisms include receptor-mediated internalization

- 209 -

by specialized cells, such as macrophages or macrophage precursors, endothelial cells lining the sinusoids of the liver, spleen, and bone marrow, and reticular cells of lymphatic tissue and of bone marrow. Examples of receptors that mediate clearance of polypeptides in the circulation include Fc- γ receptor(s), which bind IgG-antigen
5 complexes; lipoprotein receptors (e.g. LDL receptor-related protein receptor (LRP), LDL receptor and VLDL receptor); scavenger receptors (e.g. LRP, LDL-receptor, SR-A, SR-BI, CD36, etc.), which bind many different classes of serum macromolecules; hyaluronan receptors, which bind matrix proteoglycans; collagen alpha-chain receptors, which bind collagen alpha-chains; mannose receptors, which bind carboxy-terminal propeptides of
10 type I procollagen and tissue plasminogen activator; and the like. A clearance domain can thus be a ligand for a receptor that mediates clearance, such as a polypeptide or fragment thereof that binds a receptor type mentioned above.

An example of a clearance domain is a ligand for an Fc receptor. There are several Fc receptors (FcR), including Fc γ RI, Fc γ RII, Fc γ RIII, and the neonatal Fc
15 receptor (FcRn), which bind IgG antibodies. An Fc receptor ligand can be the Fc portion of an IgG (i.e. the portion containing the carboxy termini of the two heavy (H) chains, when an antibody is cleaved with papain), or a fragment thereof that retains Fc receptor binding. The antibody portions involved in Fc receptor binding are known in the art or can be determined by receptor binding assays known in the art. For example, the lower
20 hinge and the adjacent region of the CH2 domain of IgG Fc are involved in binding to Fc γ RIIa, whereas the Fc CH2-CH3 interface is involved in binding to Fc γ RIIb and FcRn (Wines *et al.* (2000) *J. Immunol.* 164:5313-5318). Exemplary clearance domains are the Fc domain of an IgG1 human or an Fc domain of antibody IgG2a mouse antibody.

Another example of a clearance domain is a ligand for LRP. At least 30
25 molecules that bind LRP are known in the art, including, for example, APP, ApoE, alpha-2-macroglobulin, tPA, blood coagulation factors, lactoferrin, C1 inhibitor, pregnancy zone protein, thrombospondins, complement C3, and the like (see Herz and Strickland (2001) *J. Clin. Invest.* 108:779-784). The portions of these proteins that bind LRP are known in the art, or can be determined by LRP binding assays known in the art
30 (see, for example, U.S. Patent No. 6,472,140, which describes LRP-binding fragments of

- 210 -

alpha-2-macroglobulin that comprise residues 1366-1392 of human alpha-2-macroglobulin). Any of these molecules, or portions thereof that bind LRP, can be used as clearance domains.

Provided herein are A β binding proteins containing a clearance domain. In one embodiment, an A β binding protein comprises an A β antibody and an Fc region. The Fc region may originate from the A β antibody or the Fc domain may be from another antibody or generated synthetically and joined to the A β antibody by recombinant or chemical means. In another embodiment, an A β binding protein comprises one or more CDRs from an A β antibody and additionally, an Fc clearance domain, for example an A β binding protein containing one or more CDRs of an A β antibody grafted into a scaffold and an Fc clearance domain. In yet another embodiment, an A β binding protein comprises a clearance domain from an LRP ligand.

(d) Additional functionalities

A β binding proteins can be constructed which comprise additional functionalities such as a moiety for detection or purification of the A β binding protein, a therapeutic moiety or an additional domain such as for indirect clearance.

Detectable moieties may be associated with an A β binding protein by chemical or recombinant means. For example, a protein domain which can be detected by visible or enzymatic assay can be coupled to an A β binding protein. Example of such domains include fluorescent proteins such as green, red and blue fluorescent proteins, β -galactosidase, alkaline phosphatase and others known in the art. A radiolabel may also be coupled to an A β binding protein for example, ^{125}I , ^{131}I , ^{213}Bi , $^{99\text{m}}\text{Tc}$, ^{111}In , ^{90}Y , or ^{32}P , such as for detection, imaging, diagnostic and therapeutic purposes.

Additional functional domains can also include indirect or regulated clearance domains. For example, an A β binding protein can comprise a biotin moiety and a streptavidin molecule such as galactosylated streptavidin can be used for clearance (Govindan et al. Cancer Biother Radiopharm. 2002 Jun;17(3):307-16).

3. Characterizing A β antibodies and A β binding proteins

(a) Determination of A β Binding

Antibodies (including antibody fragments) and A β binding proteins described

- 211 -

herein can be assayed by any method known in the art for assessing binding to A β .

Methods to assess binding include assays such as ELISA, western blotting, immunoprecipitation, two hybrid assays, phage display and others well known in the art.

Binding assays can be used to ascertain if the prepared antibody or A β binding protein
5 binds to A β . Binding assays can also be used to ascertain if the antibody or A β binding protein binds selectively to a particular A β . A β antibodies and binding proteins can be tested against a specific A β to determine which are preferentially bound. Peptides tested can include deletion variants of A β , including both N and C-terminal truncations of A β , as well as deletions within the central region of the A β peptide. Such peptides can be
10 used to map the minimal amino acid sequences of A β recognized by an A β antibody or binding protein. For example, such binding assays can be used to demonstrate that the exemplary antibody A387 binds preferentially to A β 42 with minimal or no binding to other A β peptides such as A β 1-40 and A β 1-39.

Methods known in the art can also be used to ascertain the relative binding
15 affinity and avidity of the antibodies and A β binding proteins for A β and/or various forms of A β . For example, A β antibodies and binding proteins can be tested using binding assays such as ELISA, dot blots and immunoprecipitation with A β in soluble form, aggregates, low molecular weight oligomers, in plaques and neurofibrillary tangles. Such assays can be performed with isolated A β peptides or with samples taken from
20 cells and tissues such as those of cell lines, animal models and subjects. A β can be solubilized and/or aggregated using *in vitro* methods such as sonication, and fibril growth *in vitro* (O'Nuallain et al., (2002) PNAS 99(3):1485-1490). Additionally, chemical reagents, such as metal chelators, can be used to generate low molecular weight forms of A β and then used to assays to assess the reactivity of an A β binding protein or A β
25 antibody for the low molecular weight forms of A β . Assays can also be used to assess binding to specific molecular weight forms of A β such as monomers and low molecular weight oligomers or high molecular weight oligomers and aggregates. For example gel filtration and native gels can be used to assess the relative molecular weight or size of A β recognized by an A β antibody or A β binding protein. Western blotting and
30 immunoprecipitation can also be used to assess selectivity of A β binding proteins and

- 212 -

antibodies for a particular A β . For example, as described in Example 9, A β can be treated with the metal chelator bathocuprione (BC) and then reacted with an A β antibody or A β binding protein in subsequent immunoassays. Such assays can be used to screen A β antibodies and A β binding proteins to isolate those specific for binding A β and particular A β peptides in a specific form or which bind only to a particular A β in a specific form. In one embodiment, antibodies are isolated which bind only to A β in low molecular weight forms. In another embodiment, antibodies are isolated which bind to A β 42 and preferentially bind A β 42 in low molecular weight forms. An exemplary antibody which binds selectively to A β 42 and to A β 42 preferentially in low molecular weight forms is the antibody A387.

(b) Clearance properties

A β antibodies and A β binding proteins can be assessed for their rate of clearance from the circulation using *in vivo* pharmacokinetic assays and/or *in vitro* assays that sufficiently correlate with *in vivo* results. Such assays are well known in the art (see, for example, Shargel and Yu (1999) "Applied Biopharmaceutics and Pharmacokinetics," 4th ed., McGraw-Hill/Appleton & Lange). For example, suitable assays can assess the half-life of the binding protein or antibody, and/or of bound A β , in cell-culture medium or blood; the uptake of the binding protein or antibody, and/or of bound A β , by a cell, tissue or organ; the intracellular or extracellular accumulation of degradation products of the binding protein or antibody; and the like.

In one type of *in vivo* assay, a detectably labeled (e.g. radiolabeled) A β binding protein or antibody is administered to a subject, and the decreasing level of label in the circulation, or the increasing level of label in the urine or liver, is monitored to assess the rate of clearance of the A β binding protein or antibody from the circulation. In another type of *in vivo* assay, an unlabeled A β binding protein or antibody is injected to a subject, and at various times after dosing, plasma is collected. Various assays can then performed to determine the concentration of administered protein remaining in the circulation. For example, an ELISA assay can be performed, using suitable capture reagents (e.g. A β) and detection reagents (e.g. a labeled secondary antibody). Alternatively, a radioimmunoassay (RIA) can be performed, in which the plasma A β binding protein or

- 213 -

antibody competes for binding of radiolabeled A β binding protein or antibody to a suitable secondary reagent.

In one type of *in vitro* assay, the uptake of detectably labeled A β binding protein or antibody from the culture medium by cells having receptors for the clearance domain is assessed. For example, if the clearance domain is a ligand for an Fc receptor, the cells can be macrophages. If the clearance domain is a ligand for LRP, because of the ubiquitous nature of LRP, the cells can be of essentially any tissue origin, such as hepatocytes and fibroblasts. After a suitable incubation period, cells are washed and the amount of intracellular label measured.

10 (c) Purification

A β antibody and A β binding protein purification may be carried out using standard protein purification techniques. Exemplary methods include ion exchange chromatography, HPLC, and affinity chromatography. Affinity chromatography using Protein A or Protein G can be used to purify A β antibodies and A β binding proteins with antibody scaffolds. Affinity chromatography with A β peptides can be used to purify proteins which bind A β . A β antibodies and binding proteins can be generated with purification tags, such as a His₆ tag for metal binding, to facilitate purification. Such tags can be designed to be cleaved after the affinity purification step to produce purified A β antibodies and binding proteins. Purification can be assessed by standard methods known in the art such as electrophoresis and staining and mass spectrometry.

4. Expression of A β binding proteins

Numerous techniques are known in the art for the design of constructs to express A β binding proteins including A β antibodies and/or portions thereof. Expression constructs can be used for expression, for example, *in vitro* or *in vivo*, in cells, extracts, tissues or whole organisms. Such constructs are useful for assessing properties of A β binding proteins. Additionally, expression constructs are useful in the production of cell lines and transgenic organisms expressing A β binding proteins, including those used in screening methods described herein and known in the art.

a. Vectors and Constructs

30 A vector will generally contain elements useful for cloning and/or expression of

- 214 -

inserted nucleic acid molecules, such as an origin of replication compatible with the intended host cells; promoter, enhancer and/or other regulatory sequences, which can provide for constitutive, inducible or cell type-specific RNA transcription; transcription termination and RNA processing signals, such as a polyadenylation signal; one or more
5 selectable markers compatible with the intended host cells (e.g. a neomycin or hygromycin resistance gene, useful for selecting stable or transient transfectants in mammalian cells, or an ampicillin or tetracycline resistance gene, useful for selecting transformants in prokaryotic cells); and versatile multiple cloning sites for inserting nucleic acid molecules of interest. The choice of particular elements to include in a
10 vector will depend on factors such as the intended host cells, the insert size, whether expression of the inserted sequence is desired, the desired copy number of the vector, the desired selection system, and the like. Vectors suitable for use in cloning and expression applications include, for example, viral vectors such as a bacteriophage, adenovirus, adeno-associated virus, herpes simplex virus, vaccinia virus, baculovirus and retrovirus;
15 cosmids or *Escherichia coli*-derived, *Bacillus subtilis*-derived and yeast-derived plasmids; bacterial artificial chromosome vectors (BACs) and yeast artificial chromosome vectors (YACs). Such vectors and their uses are well known in the art.

Nucleotide sequences that can be used to express proteins generally contain one or more transcriptional regulatory sequences (e.g. promoters, enhancers, terminators and
20 the like) in operative association with the expressed sequence (e.g. an A β binding protein or portion thereof). Promoters for gene expression regulation include, for example, promoters for genes derived from viruses (e.g., cytomegalovirus (CMV), Moloney murine leukemia virus (MMLV), JC virus, rous sarcoma virus (RSV), simian virus SV40, mouse mammary tumor virus (MMTV), *etc.*), promoters for prokaryotic
25 expression such as T3 and T7 promoters, and promoters for genes derived from various mammals (e.g., humans, rabbits, dogs, cats, guinea pigs, hamsters, rats, mice *etc.*) and birds (e.g., chickens *etc.*) (e.g., genes for albumin, insulin II, erythropoietin, endothelin, osteocalcin, muscular creatine kinase, platelet-derived growth factor beta, keratins K1, K10 and K14, collagen types I and II, atrial natriuretic factor, dopamine beta-
30 hydroxylase, endothelial receptor tyrosine kinase (generally abbreviated Tie2), sodium-

- 215 -

potassium adenosine triphosphorylase (generally abbreviated Na,K-ATPase), neurofilament light chain, metallothioneins I and IIA, metalloproteinase I tissue inhibitor, MHC class I antigen (generally abbreviated H-2L), smooth muscle alpha actin, polypeptide chain elongation factor 1 alpha (EF-1 alpha), beta actin, alpha and beta
5 myosin heavy chains, myosin light chains 1 and 2, myelin base protein, serum amyloid component, myoglobin, renin *etc.*). Inducible promoters such as chemically inducible promoters, for example, regulated by tetracycline, or steroids such as ecdysone, estrogen, or progesterone and others known in the art, may be used for expression.

The above-mentioned vectors can have a sequence for terminating the
10 transcription of the desired messenger RNA in the transgenic animal (generally referred to as terminator); for example, gene expression can be manipulated using a sequence with such function contained in various genes derived from viruses, mammals and birds. The simian virus SV40 terminator and other known terminators known in the art are commonly used. Additionally, for the purpose of increasing the expression of the desired
15 gene, various other elements may be included: *e.g.*, the splicing signal and enhancer region of each gene, a portion of the intron of a eukaryotic organism gene may be ligated 5' upstream of the promoter region, or between the promoter region and the translational region, or 3' downstream of the translational region as desired.

A β binding proteins can be expressed as a single expression construct or may be
20 expressed as multiple expression constructs. For example, an A β antibody comprised of a heavy and light chain can be produced by constructing an expression construct for heavy chain expression and a second expression construct for light chain expression. The two expression constructs may be contained on the same vector or on two separate vectors. They can be integrated together into a host cell or organism or alternatively
25 integrated at different locations.

b. Cell culture production

A β binding proteins including A β antibodies and fragments thereof can be expressed in cell culture as a means of producing them for use in diagnostics, research or treatment. Expression in cell culture can also be used as the basis for characterizing and
30 testing A β binding proteins and for further screening assays to identify molecules which

- 216 -

modulate or alter the interaction between A β binding proteins and A β .

Nucleic acid molecules can be introduced into host cells by various well-known transfection methods, including electroporation, infection, calcium phosphate co-precipitation, protoplast or spheroplast fusion, lipofection, micro-injection, and DEAE-dextran-mediated transfection (*e.g.* Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 2nd ed., Cold Spring Harbor Press, Plainview, N.Y. (1989); Ausubel et al., *supra*, (1999), Keown *et al.* (1990) *Methods in Enzymology* 185:527-537). Host cells can be maintained and propagated by methods known in the art (*e.g.* Freshney, R. I. (2000) "Culture of Animal Cells: A Manual of Basic Technique," 4th ed., Wiley-Liss).

Any cell line known in the art to be suitable for protein and/or antibody production can be used to produce A β binding proteins. Suitable host cells include human and other mammalian cells, including primary cells and cell lines. Exemplary host cells include mammalian primary cells (*e.g.* cells from any tissue of human, rabbit, dog, cat, guinea pigs, hamsters, rats, mice, etc.); embryonic stem cells, fertilized eggs and embryos; myeloma cells, cells contained in, or obtained from, transgenic animals; established mammalian cell lines, such as SY5Y, RBL, COS, CHO, HeLa, NIH3T3, HEK 293, BHKBI and Ltk⁻ cells, mouse monocyte macrophage P388D1, J774A-1 and PC12 cells (available from ATCC, Manassas, VA); amphibian cells, such as *Xenopus* embryos and oocytes; avian cells; and other vertebrate cells. Exemplary host cells also include insect cells (*e.g.* *Drosophila*), yeast cells (*e.g.* *S. cerevisiae*, *S. pombe*, *Candida tropicalis*, *Hansenula polymorph* or *Pichia pastoris*), plant cells and bacterial cells (*e.g.* *E. coli*).

In some cases it may be desirable to modify the expressed proteins. In vitro can be used to accomplish modifications such as glycosylation, for example galactosylation and sialylation (Raju et al. *Biochemistry*. 2001 Jul 31;40(30):8868-76). Alternatively, in vivo modification can be accomplished by expression in cell lines which carry out such modifications or by the engineering of cell lines to provide the appropriate modifications (Choi, et al. *Proc. Natl Acad Sci U S A*. 2003 Apr 29;100(9):5022-7. Epub 2003 Apr 17).

Cell lines for or characterizing and testing A β binding proteins and for further

- 217 -

screening assays typically include cell lines that produce A β , for example primary cell cultures, typically neuronal cell cultures. Totipotent, pluripotent, or other cells that are not terminally differentiated can be induced to express neuronal characteristics including the production of A β peptides. Exemplary non-terminally differentiated cells include

5 embryonic stem cells, adult stem cells, mesenchymal stem cells, bone marrow stem cells, adipose tissue stem cells, and neuronal stem cells. Additionally, cells can be engineered to express forms of A β or fragments thereof. Examples of such cell cultures, methods for induction of A β production, harvesting and culturing are described herein. A β binding proteins including A β antibodies can be added exogenously to cells expressing A β or

10 expression of the A β binding proteins can be engineered within the same cell.

Nucleic acid encoding A β binding protein and A β antibody or portion thereof may be stably incorporated into cells or may be transiently expressed using methods known in the art. Stably transfected cells may be prepared by transfecting cells with an expression vector having a selectable marker gene (such as, for example, the gene for

15 thymidine kinase, dihydrofolate reductase, neomycin resistance, and the like), and growing the transfected cells under conditions selective for cells expressing the marker gene. Transient expression may use similar methods without selectable markers or may use viral expression such as baculovirus, vaccinia virus, adenovirus and other transient systems known in the art.

20 Heterologous nucleic acid may be maintained in the cell as an episomal element or may be integrated into chromosomal DNA of the cell. The resulting recombinant cells may then be cultured or subcultured (or passaged, in the case of mammalian cells) from such a culture or a subculture thereof. Methods for transfection, injection and culturing recombinant cells are known to the skilled artisan. Expression of an A β binding protein

25 mRNA or protein in cells can be assessed by methods known in the art such as Northern blotting, RT-PCR, Taqman, Western Blotting, ELISA, enzymatic function of an A β binding protein, and binding or interaction properties of an A β binding protein. Methods for protein expression and purification are known in the art (see, for example, Sambrook et al. (1989) MOLECULAR CLONING: A LABORATORY MANUAL 2nd

30 Ed. Cold Spring Harbor Laboratory Press; Ausubel et al. (1995) CURRENT

- 218 -

PROTOCOLS IN MOLECULAR BIOLOGY, Greene Publishing Co., NY; Rosenberg, I.M. (1996) "Protein Analysis and Purification: Benchtop Techniques" *Springer Verlag*; and Scopes, R.K. (1994) "Protein Purification: Principles and Practice" *Springer Verlag*.)

Biological compositions can be derived from cell lines such as but are not limited to, purified or partially purified enzyme preparations, conditioned medium from cultured cells, cellular extracts and cell lysates. Such compositions can be generated using methods described herein and/or known in the art for use in characterizing A β binding proteins and for further screening assays.

c. Transgenic Animals

Also provided herein are methods of producing transgenic animals by introducing nucleic acid encoding an A β binding protein into a cell and allowing the cell to develop into a transgenic animal. The cell may be any cell that may be used in the generation of a transgenic animal. Such cells are known to those of skill in the art of transgenic animal production. For example, the cell may be an embryo, zygote, oocyte, fertilized oocyte or embryonic stem cell, such as, for example, a mouse embryonic stem cell. Numerous techniques for introduction of exogenous nucleic acids into cells that will be allowed to develop into transgenic animals are also known to those of skill in the art. Such techniques include, but are not limited to, pronuclear microinjection (see, *e.g.*, U.S. Patent No. 4,873,191), retrovirus-mediated gene transfer into germ lines [see, *e.g.*, Van der Putten *et al.* (1985) *Proc. Natl. Acad. Sci. U.S.A.* 82:6148-6152], gene targeting into embryonic stem cells [see, *e.g.*, Thompson *et al.* (1989) *Cell* 56:313-321], electroporation of embryos [see, *e.g.*, Lo (1983) *Mol. Cell. Biol.* 3:1803-1814], and sperm-mediated gene transfer [see, *e.g.*, Lavitrano *et al.* (1989) *Cell* 57:717-723] [for a review of such techniques, see Gordon (1989) *Int. Rev. Cytol.* 115:171-229]. A cell into which exogenous nucleic acid has been transferred may be introduced into a recipient female animal for development into a transgenic animal containing the exogenous nucleic acid.

Methods for making transgenic animals using a variety of transgenes have been described [see, *e.g.*, Wagner *et al.* (1981) *Proc. Nat. Acad. Sc. U.S.A.* 78:5016; Stewart *et al.* (1982) *Science* 217:1046; Constantini *et al.* (1981) *Nature* 294:92; Lacy *et al.* (1983)

- 219 -

Cell 34:343; McKnight *et al.* (1983) *Cell* 34:335; Brinster *et al.* (1983) *Nature* 306:332; Palmiter *et al.* (1982) *Nature* 300:611; Palmiter *et al.* (1982) *Cell* 29:701, and Palmiter *et al.* (1983) *Science* 222:809; Ono *et al.* (2001) *Reproduction* 122:731-736; Reggio *et al.* (2001) *Biol. Reprod.* 65:1528-1533; Park *et al.* (2001) *Animal Reprod. Sci.* 68:111-120; 5 Zakhartchenko *et al.* (2001) *Mol. Reprod. Dev.* 60:362-369; Arat *et al.* (2001) *Mol. Reprod. Dev.* 60:20-26; Koo *et al.* (2001) *Mol. Reprod. Dev.* 58:15-20; Polejaeva and Campbell (2000) *Theriogenology* 53:117-126]. Such methods are also described in U.S. Patent Nos. 6,175,057; 6,180,849 and 6,133,502, 6,271,436, 6,258,998, 6,103,523, 6,252,133.

10 **d. *In vitro* and Synthetic systems**

A β antibodies and A β binding proteins and fragments thereof can be produced *in vitro* in cell-free systems (Makeyev *et al.* (1999) *FEBS Lett.* 444:177-180). Such systems can be useful for rapid screening of constructs and mutants to ascertain function and binding specificity. For example, expressible antibodies and binding proteins can be 15 constructed using PCR techniques to join a T7 or other known RNA polymerase tag onto the nucleotide sequence encoding the polypeptide. *In vitro* transcription and translation can then be used to express the polypeptides for use in binding or other assays. Single antibodies or binding proteins or libraries of such polypeptides can be produced by such methods.

20 Synthetic means can also be used to produce A β antibodies and A β binding proteins. For example, regions of A β antibodies and A β binding proteins can be synthesized *in vitro* and joined to scaffold molecules. Peptides of one or more CDRs of an A β antibody can be synthesized and tested for reactivity with A β .

25 **J. Treatment of Disease and Disorders with A β binding proteins**

Methods are provided herein for the use of A β binding proteins and A β antibodies in the treatment or prophylaxis of diseases involving or characterized by A β and/or specific A β forms. . Such diseases include, but are not limited to, diseases involving or associated with amyloidosis and neurodegenerative diseases. One example 30 of such a disease is Alzheimer's disease.

- 220 -

Genetic and biochemical evidence indicates that accumulation of A β is involved in the pathogenesis of Alzheimer's and further that specific forms of A β , such as accumulation into oligomers, aggregates and plaques, participates in the pathogenesis of the disease. Immunization with A β peptides as well as passive immunization with A β antibodies has been shown to modulate both A β levels and related pathogenic and behavioral effects (Holtzman et al. (2002) Adv. Drug Delivery Rev. 54:1603-1613; Dodart et al., (2002) Nature Neurosci. 5(5):452-457; Bard et al., (2003) PNAS 100(4):2023-2028; WO00/72880). The methods are suitable for the treatment or prevention of disease because they are designed to selectively modulate A β levels.

10 Methods herein are also provided to modulate the level of a particular A β , such as A β 42.

Methods herein can include a step of administering an A β binding protein or A β antibody to a subject having such a disease or disorder or predisposed to such a disease or disorder. In one embodiment of the methods, the A β binding protein or A β antibody being administered is one that modulates the level of one or more A β peptides. In one embodiment, A β 42 levels are modulated. The level of A β 42 can be modulated to a greater extent than the level of one or more other A β peptides, in particular, A β 40, such that the level of A β 42 is modulated, or without substantially altering the level of one or more other A β peptides, in particular A β 40. In a particular embodiment, A β 42 levels are reduced.

15

20

In one embodiment, an A β binding protein or A β antibody being administered is one that preferentially binds a specific form of A β such as A β in low molecular weight forms. In one aspect of the embodiment, the A β binding protein or A β antibody is specifically reactive with a specific A β , in particular A β 42, and also preferentially binds low molecular weight forms of A β 42. In a particular embodiment, the A387 antibody or a fragment thereof is administered. In another embodiment, an A β binding protein which retains the binding specificity of the A β antibody for low molecular weight forms of A β 42 is administered. For example, a humanized antibody that preferentially binds low molecular weight forms of A β 42 is administered. In one embodiment, an antibody containing the sequence of SEQ ID NO:12 and and/or SEQ ID NO:14, or portion

25

30

- 221 -

thereof, is administered.

In another embodiment, an A β binding protein or A β antibody being administered is one that recognizes the N-terminal region of A β . In a particular embodiment, the B436 antibody, or a fragment thereof is administered. In another embodiment, an A β binding
5 protein which retains the binding specificity of the B436 antibody for N-terminal region of A β is administered. For example, a humanized antibody which retains the binding specificity of B436 is administered. In one embodiment, an antibody containing the sequence of SEQ ID NO:16 and/or SEQ ID NO:18, or portion thereof, is administered.

1. Predictive assays

10 A β binding assays such as those described herein and known in the art can be used to assess the reactivity of A β antibodies and A β binding proteins with A β . Determination of specificity, affinity, avidity as well as stability and clearance can assist in determining dosages and administration regimes. Assessment of the binding properties of A β antibodies and A β binding proteins can be ascertained for binding to
15 specific forms of A β such as binding to A β in soluble or aggregate forms, binding of monomers, low molecular weight oligomers or high molecular weight aggregates. Assays such as those described herein for assessing binding to A β and specific A β peptides and forms of A β , and assays for clearance as well as additional methods known in the art can be used for assessing A β antibodies and binding proteins.

20 Animal models can also be used for the assessment of A β antibodies and A β binding proteins for the treatment of diseases and disorders associated with A β for example with altered A β levels, and/or altered ratios of one or more A β peptides and/or forms. In particular, non-human animals that have altered production, degradation and/or clearance of A β peptides or altered expression of APP can be used for such assays.

25 Examples of such animals include transgenic animal models and animals, such as rodents, including mice and rats, cows, chickens, pigs, goats, sheep, monkeys, including gorillas, and other primates. Exemplary animal models include animals with the Swedish mutation of APP (Asp595-leu596), disclosed in US Patent Nos. 5,612,486 and 5,850,003, the transgenic mouse disclosed in US Patent No. 5,387,742, which expresses
30 particular APP species that form β -amyloid protein deposits in the brain of the mouse,

- 222 -

and TASD41 transgenic mice, which express human APP751 cDNA containing the London (V717I) and Swedish (K670M/N671L) mutations under the control of the murine Thy-1 gene (Rockenstein *et al.* (2001) *J. Neurosci. Res.* 66:573-582). Additional transgenic animal models include those described in US Patent Nos. 5,811,633; 5 6,037,521; 6,184,435; 6,187,992; 6,211,428; and 6,340,783, transgenic mouse models Tg 2576; APPSWE mouse, K670N, M671L, and other models including APP(V717F), APP(K670N, M671L and V717F), PS-1 M146L, PS-1 M146V, APPSWE + PS A246E (reviewed by Emilien, *et al.*, (2000) *Arch. Neuro.* 57: 176-81).

A β antibodies and A β binding proteins can be administered, such as by injection, 10 to animal models and the effects of such treatment assessed. For example, animals can be injected one or more times intraperitoneally, or by other suitable route, with an A β antibody or A β binding protein. Alternatively, transgenic expression can be used to produce an A β antibody or A β binding protein in an animal and the effects are assessed in the animal. For example, an A β antibody or A β binding protein can be expressed in a 15 wildtype animal model and the animal is then assessed. An A β antibody or A β binding protein can also be expressed in a model animal for a disease or condition.

2. Administration of antibodies to subjects

A β antibodies and A β binding proteins can be administered to subjects for prophylactic and therapeutic uses. In prophylactic applications, a composition or 20 medicament is administered to a subject at risk for a disease or condition such as Alzheimer's disease. In therapeutic treatments, a composition or medicament is administered to a subject suspected of or already suffering from a disease or condition, such as Alzheimer's disease. An amount of the composition or medicament is administered to achieve an effectiveness of treatment. As described herein, predictive 25 assays such as in vitro and in vivo assays, including testing in animal models can be used to determine dosages and dosage regimes for treatment.

Dosages of A β antibodies and A β binding proteins for treatment will vary depending on conditions such as the means of administration, the target site, the species of subject and physiological state of the subject and the use of the treatment (e.g. 30 prophylactic or therapeutic). Treatment dosages are optimized for safety and

- 223 -

effectiveness. Dosages range from 0.0001 to 100 mg/ kg of subject body weight. Typically, dosages are 0.01 to 10 mg/kg. In some cases, more than a single dose of the composition or medicament is necessary to achieve an effectiveness of treatment. For example, dosages can be daily, weekly, monthly or yearly. Dosages and dosage regimes
5 can be determined empirically for example, by measuring the levels of A β , specific A β peptides and/or forms, and achieving a desired level of such in the subject by administering an A β antibody or A β binding protein to maintain that level. The dosages and dosage regimes can also depend on the stability of an A β antibody or A β binding protein. Stability of an A β binding protein or antibody can be determined by measuring
10 levels of the protein or antibody in *in vitro* assays, cell based assays, in animal models and in a subject. For example, an amount of an A β antibody or protein can be administered to a subject and subsequent samples, such as blood, plasma or cerebral spinal fluid samples, taken from the subject over time to assess the amount remaining in the subject. In some cases an A β antibody or an A β binding protein with a detectable
15 moiety such as a radiolabel, may be used to facilitate measurements.

A β antibodies and A β binding proteins can be administered by parenteral, topical, intravenous, oral, subcutaneous, interarterial, intracranial, intraperitoneal, intranasal and intramuscular means. A β antibodies and A β binding proteins can be administered to a particular organ or tissue, for example, by injecting directly into the organ or tissue. For
20 example, A β antibodies and A β binding proteins can be injected directly in the cranium, into a muscle and directly into the bloodstream. For administration, A β antibodies and A β binding proteins can be formulated as a solution or suspension in a physiological diluent such as sterile water, saline, glycerol, oil or ethanol. Formulations can also be prepared as liposomes or micelles, microparticles and in formulation for sustained
25 release. Formulations can also include surfactants, emulsifying agents, wetting agents, and pH buffering substances.

A β antibodies and A β binding proteins can also be administered in combination with other treatments, for example in combination another treatment for the disease or condition. For example, an A β antibody can be administered along with an agent that
30 modulates the processing or levels of APP for treatment of Alzheimer's disease.

- 224 -

3. Assessment of Treatment

Methods for assessing treatment can be biochemical, physiological and/or can involve assessments of behaviors or phenotypes associated with a particular condition or disease. The effectiveness of treatment can include the effectiveness of a treatment to
5 ameliorate symptoms such as by decreasing the severity, delaying the onset, delaying the recurrence, or decreasing the number of recurrences of symptoms or by delaying the progression of a disease or condition. Effectiveness of treatment also can include the effectiveness of a treatment to prevent a disease or condition, prevent the onset of symptoms of disease or condition. The effectiveness of ameliorating or preventing
10 symptoms and/or the occurrence of a condition or disease can be assessed in animals, animal models and/or in subjects.

(a) Biochemical and Physiological Phenotypes

Levels and forms of A β can be observed after to treatment to ascertain changes in the levels of A β , such as levels of all A β peptides, levels of particular A β peptides, such
15 as A β 42, and changes in the form of A β , for example, the level of soluble A β and the level in plaques.

A β can be assessed in plasma for example after treatment and obtaining blood at sacrifice from animals by cardiac puncture. Blood is then centrifuged to obtain plasma which can then be tested for A β levels and forms by assays such as described herein or
20 known in the art. For example, A β levels can be assed in an ELISA assay with A β antibodies. Additionally, the plasma can be tested for the level of treatment agent. For example, A β antibodies and/or A β binding proteins present in the sample can be detected by biochemical and/or immunological means. Levels and forms of A β can also be assessed in cerebrospinal fluid in a similar manner. A β can also be assessed in tissues
25 such as the brain for example, by obtaining brain tissue from each animal at sacrifice. As described in the Examples herein, homogenates of brain sections can be analyzed for A β levels by ELISA or by other assays described herein or known in the art to assess A β levels and forms. Additional dissection into cortex, hippocampus and cerebellar regions before homogenization can be used to further localize A β .

30 Histopathology can also be used to assess treatment .For example as described in

- 225 -

the Examples, brain sections can be assayed for the abundance of amyloid plaques in treated and control animals. *In situ* analysis with antibody staining can also be used to ascertain levels of A β and A β forms, for example by using A β antibodies which recognize A β and/or specific A β forms (Dodart *et al.* (2002) *Nature Neurosci.* 5(5): 452-
5 257). An A β antibody or A β binding protein with a detectable moiety can be used to detect the presence, level, stability and/or localization of the administered A β antibody or A β binding protein. For example, an initial dose of an A β antibody or A β binding protein with a detectable moiety can be administered and the level, stability and/or localization assessed to determine further dosing in the same animal or subject or to
10 assist in predicting the dosage for additional animals or subjects to be treated.

(b) Behavioral Phenotypes

Behavioral phenotypes specific for an A β -associated condition or disease can be measured to ascertain the effect of treatment. For example, an assessment of Alzheimer's disease (AD) phenotype can refer to any visible, detectable or otherwise measurable
15 symptom or property of an individual diagnosed with AD. Such properties include, but are not limited to, dementia, aphasia (language problems), apraxia (complex movement problems), agnosia (problems in identifying objects), progressive memory impairment, disordered cognitive function, altered behavior, including paranoia, delusions and loss of social appropriateness, progressive decline in language function, slowing of motor
20 functions such as gait and coordination in later stages of AD, amyloid-containing plaques, which are foci of extracellular amyloid beta protein deposition, dystrophic neurites and associated axonal and dendritic injury, microglia expressing surface antigens associated with activation (*e.g.*, CD45 and HLA-DR), diffuse ("preamyloid") plaques and neuronal cytoplasmic inclusions such as neurofibrillary tangles containing
25 hyperphosphorylated tau protein or Lewy bodies (containing α -synuclein). Standardized clinical criteria for the diagnosis of AD have been established by NINCDS/ADRDA (National Institute of Neurological and Communicative Disorders and Stroke/Alzheimer's Disease and Related Disorders Association) (McKham *et al.* (1984) *Neurology* 34:939-944). The clinical manifestations of AD as set forth in these criteria
30 are included within the definition of AD phenotype. For example, dementia may be

- 226 -

established by clinical exam and documented by any of several neuropsychological tests, including the Mini Mental State Exam (MMSE) (Folstein and McHugh (1975) *J. Psychiatr. Res.* 12:196-198; Cockrell and Folstein (1988) *Psychopharm. Bull.* 24:689-692), the Blessed Test (Blessed *et al.* (1968) *Br. J. Psychiatry* 114:797-811) and the
5 Alzheimer's Disease Assessment Scale-Cognitive (ADAS-COG) Test (Rosen *et al.* (1984) *Am. J. Psychiatry* 141:1356-1364; Weyer *et al.* (1997) *Int. Psychogeriatr.* 9:123-138; and Ihl *et al.* (2000) *Neuropsychobiol.* 4:102-107).

Tests can be developed in suitable laboratory animals to assess the effects of a treatment. For example, in AD, AD model animal can be treated and assessed. In one
10 example, an object recognition task can be used to assess treatment. The test is based on the animal's spontaneous tendency to explore a novel object more frequently than a familiar one (Ennaceur *et al.* (1988) *Behav. Brain Res.* 31:47-59; Dodart *et al.* (1997) *Neuroreport* 8:1173-1178). Briefly, an animal such as a mouse is tested in a first trial with an object (such as a marble) and then in a second trial with the first object plus a
15 new object (such as a die). A recognition index is calculated based on the amount of time the animal spends with each object in the second trial when both objects are present and the distance traveled toward each object.

Another example of a phenotypic test for AD is the holeboard memory task. (Dodart *et al.* (2002) *Nature Neurosci.* 5(5): 452-257). The test measures the ability of
20 an animal to remember which holes of a holeboard have been baited with food. A food pellet is placed a hole of the board and the animal is tested in several trials over consecutive days where the same hole is baited each time. A global measure of cognitive performance is calculated from the trials based on the average number of errors made by the animal each day (based on entering holes never baited, re-entering a baited hole and
25 not entering a baited hole).

Tests such as the object recognition task, holeboard memory task and other phenotypic assays known in the art are generally done with several animals to gather an average value. Single animals or groups of animals can undergo one or more treatments with a test agent, an A β binding protein, A β antibody, or any combination thereof and
30 then treatment can be assessed with a phenotypic test. Control animals which have not

- 227 -

undergone any treatments or which have undergone placebo treatments can be compared to assess the effectiveness of a particular treatment relative to no treatment or placebo controls.

The following examples are included for illustrative purposes only and are not
5 intended to limit the scope of the invention.

EXAMPLE 1

Production of A β 42-selective antibody (A387)

A selective A β 42 antibody was produced by designing a peptide with the
10 following sequence C-MVGGVVIA, which represents the A β ₃₅₋₄₂ region with N-terminal cysteine added for conjugation to ovalbumin. Swiss-Webster mice were immunized with 1 mg of the conjugated peptide followed by three boosts of 0.5 mg antigen every three weeks. Following a third boost, spleens of these mice were fused to mouse B-cells. Hybridoma cells were cloned and screened for A β 42 selectivity by
15 determining antibody titer to both the A β 40 and A β 42 peptide (AnaSpec, Inc. San Jose, CA) by ELISAs (as described below). Positive clones which had selective reactivity to the A β 42 peptide were chosen. The cells were then injected intraperitoneally into SCID mice and ascites fluid was obtained and purified using Protein A. Titer of antibodies produced was determined by coating 50 μ l of A β peptide (AnaSpec, Inc, San Jose, CA)
20 in PBS (500 ng/ml) on CoStar 3590 microtiter 96-well plates. Wells were blocked with 200 μ l of 3% BSA/PBS (Sigma, St. Louis, MO) and incubated with antibody for 1 hour at room temperature. Wells were washed three times with 200 μ l of PBS/0.1% Tween-20 (Sigma, St. Louis, MO). After washing, wells were incubated with mouse:horseradish peroxidase (HRP) secondary antibody for 1 hour at room temperature. Wells were
25 washed three times with 200 μ l of PBS/0.1% Tween-20. 50 μ l TMB (3,3',5,5'-tetramethylbenzidine) substrate was then added according to manufacturer's recommendations (KPL, Gaithersburg, MD) and incubated for 15 min. The reaction was stopped with 50 μ l of 9.8% phosphoric acid (Milwaukee, WI) and the absorbance at 450 nm was quantitated by a Biorad[®] 96-well plate reader. One antibody, designated A387,
30 was found to have >1000 fold specificity for A β 42 versus A β 40 with a very high titer as

- 228 -

determined in the above ELISA. Additionally, this antibody was shown to be specific for A β 42 versus other AB peptides; A β B1-11, 1-28, 1-38, and 1-39 when tested in the above assay. Antibody A387 was subtyped and confirmed to be IgG2a kappa. This antibody was then used to develop an A β 42 assay to quantitate A β 42 peptide produced by cells.

5

EXAMPLE 2

Production of A β 1-12 antibody (B436)

An antibody that recognizes the amino-terminal 1-12 amino-acid region on A β was produced and conjugated to alkaline phosphatase for use as a detection antibody in the A β 42 sandwich ELISA. The A β 1-12 antibody was produced by designing a peptide with the following sequence DAEFRHDSGYEV-C that represents the A β 1-12 region
10 with a C-terminal cysteine added for conjugation to ovalbumin. Swiss-Webster mice were immunized with 1 mg of the conjugated peptide followed by three boosts of 0.5 mg. Following a third boost, spleens of these mice were fused to mouse B-cells. Hybridoma cells were cloned and screened for A β reactivity. The cells were then injected
15 intraperitoneally into SCID mice and ascites was obtained and purified using Protein A. One antibody, designated B436, was found to have high titer for both A β 40 and A β 42 peptides, this was a desired feature since this antibody should equally react to any A β peptide which contains the 1-12 amino-terminal portion of the peptide. This antibody was subtyped and confirmed to be IgG2a kappa and was further purified by affinity
20 chromatography on an A β 1-12:Sephrose column and then conjugated to alkaline phosphatase. This antibody was then used as the detection antibody in the development of the A β 42 assay to quantitate A β 42 peptide produced by cells.

EXAMPLE 3

25 Production of LRP polyclonal antibody (R9377) for detection of LRP C-terminal fragments

A polyclonal antibody that recognizes the C-terminal region on LRP designated R9377 was prepared to the carboxyl-terminal 13 amino acid peptide (C-GRGPEDEIGDPLA) of LRP which was conjugated to ovalbumin via an amino-terminal cysteine residue incorporated into the LRP peptide. Initially, rabbits were primed with
30 Complete Freund's adjuvant then immunized 14 days later with 1 mg of conjugated

- 229 -

antigen and Incomplete Freund's adjuvant. Following this immunization, the rabbits received monthly boosts of antigen/Incomplete Adjuvant (0.5 mg). 14 days following the third boost, serum was collected and IgG was purified using Protein A:Sepharose. The purified antibody was used in the immunoblotting experiments described in Example 8.

5

EXAMPLE 4

A β 42 (A387) and A β 1-12 (B436) monoclonal antibody cDNA sequencing

Protocol

(1) RNA Extraction

One confluent plate (approximately 1.5×10^7 cells) each of A387 and B436 A-beta
10 mAb cell lines was harvested, pelleted, washed in 1X PBS, quick-frozen, and stored at -
80°C. Using the RNeasy Mini Kit (QIAGEN #74104) according to manufacturer's
protocol, the cells were lysed, homogenized by vortexing, and total RNA was extracted
from half of each lysate.

(2) cDNA Synthesis

15 First-strand cDNA synthesis was performed using the SuperScript First Strand
cDNA Synthesis System for RT-PCR (Invitrogen #11904-018) with antisense primers
specific for *Mus musculus* kappa light chain and IgG_{2a} heavy chain sequences (GenBank
accession numbers D14630 and V00765, respectively). The antisense primer sequences
are as follows: light chain, 5'-GGACGCCATTTTGTCTGTTCACTGCCA-3'
20 (Kappa_LCC; SEQUENCE ID NO: 22); heavy chain, 5'-
TGTTGTTTTGGCTGAGGAGACGGTGA-3' (IgG2a_HCC; SEQUENCE ID NO. 23).
Duplicate reactions containing 2.5 μ g A387 or B436 total RNA were prepared with or
without reverse transcriptase (+RT and -RT, respectively) according to the
manufacturer's protocol.

25

(3) PCR

DNA encoding the A387 and B436 light and heavy chain variable regions were
amplified by touchdown polymerase chain reaction using the Expand High Fidelity
System (Roche #1732641), degenerate sense primers, and the Kappa_LCC and
IgG2a_HCC antisense primers. The sense primers were designed using the sequence of
30 12-15 N-terminal residues from each heavy and light chain, previously obtained by N-

- 230 -

terminal amino acid sequencing performed according to standard procedures by the Protein Core Facility at the University of Nebraska on a fee for service basis. These sequences were back-translated using Vector NTI 7 software (Informax, Inc.), reducing the level of degeneracy by applying a human codon preference table. The sense primer

5 sequences are as follows: A387 light chain 5'-
 GAYATYGTSCTSACNCAGWSBCCNGC-3' (A387_LCV1; SEQUENCE ID NO. 24)
 A387 heavy chain, 5'-GARGTYAAGYTBGTYGARTCYGGAGG-3' (A387_HCV1;
 SEQUENCE ID NO: 25); B436 light chain, 5'-GAYGTYTBTATGACYCARACYCCA-
 3' (B436_LCV1; SEQUENCE ID NO: 26)); and B436 heavy chain, 5'-
 10 GARGTYATGYTBGTYGARTCYGGAGG-3' (B436_HCV1; SEQUENCE ID NO. 27).
 Reaction mixtures were prepared according to the manufacturer's protocol for each A387
 or B436 +RT and -RT reaction. Amplification was performed in a Perkin-Elmer 3700
 thermocycler according to the following conditions: denaturation for 2 min at 94°C; 10
 cycles of 15 sec at 94°C, 1 min at 70°C-0.5°C per cycle, 1 min at 72°C; 10 cycles of 15
 15 sec at 94°C, 1 min at 65°C, 1 min at 72°C; 25 cycles of 15 sec at 94°C, 1 min at 65°C, 1
 min +5 sec/cycle at 72°C; and a final extension for 7 min at 72°C.

(4) Cloning

PCR products were analyzed by gel electrophoresis on a 1% agarose gel. A
 major band of the approximate expected size (light chain: ~487 bp; heavy chain: ~408
 20 bp) was observed in each +RT reaction. An additional approximately 300-bp band was
 observed in the B436 reaction. No products were detected in the corresponding -RT
 control reactions. The desired ~487-bp and ~408-bp bands were purified using the
 QIAquick Gel Extraction Kit (QIAGEN #28704) according to the manufacturer's
 protocol. The TOPO TA Cloning Kit (Invitrogen #K4600-01) was used to clone each
 25 product into vector pCR®II-TOPO and transform E. coli TOP10 cells, according to the
 manufacturer's protocol. PCR analysis of transformants using T7 and SP6 primers
 identified 9 putative A387 light chain and 12 each putative A387 heavy chain, B436 light
 chain and B436 heavy chain constructs. Plasmid DNA was prepared for each of these
 from bacterial cultures using the QIAprep Spin Plasmid Kit (QIAGEN #27106)
 30 according to the manufacturer's protocol.

- 231 -

(4) Sequencing

The cDNA inserts were sequenced with the ABI Prism BigDye Terminators v.3.0 Cycle Sequencing Kit (ABI #4390244) using approximately 250 ng of each plasmid and 1.6 μ M each of standard T7 and SP6 primers. The manufacturer's protocol for 20 μ l reactions was followed, except that the BigDye reagent was reduced to 2 μ l and supplemented with 4 μ l 5X Sequencing Buffer (ABI #4305603) per reaction. Reactions were purified using the CleanSEQ Kit (Agencourt #000136) according to the manufacturer's protocol then analyzed on an ABI 3700 sequencer. The results were evaluated using Sequencher software (Gene Codes Corp.). A387 light chain nucleotide sequences were obtained from seven independent clones. The identity of every nucleotide between the primer binding sites was confirmed by agreement between at least six of those sequences, with 99% of the sequence identical in all seven clones. Similarly, the identity of each nucleotide was confirmed in at least 10 of 11 A387 heavy chain clones, 11 of 12 B436 light chain clones, and 11 of 12 B436 heavy chain clones, with >99% of the sequences identical in all clones for each case. Some nucleotide sequence variability was seen in the N-terminal primer binding sites due to primer degeneracy. However, the amino acid sequences for these regions was previously determined by N-terminal amino-acid sequencing.

The nucleotide sequences obtained are provided in SEQ ID NO: 11 (A387 light chain variable region (nucleotides 1-285), J region (nucleotides 286-321) and N-terminal sequence of a constant region (nucleotides 322-478)), SEQ ID NO: 13 (A387 heavy chain variable region (nucleotides 1-291), DJ region (nucleotides 292-354) and N-terminal sequence of a constant region (nucleotides 355-366)), SEQ ID NO: 15 (B436 light chain variable region (nucleotides 1-300), J region (nucleotides 301-336) and N-terminal sequence of a constant region (nucleotides 336-493)), SEQ ID NO: 17 (B436 heavy chain variable region (nucleotides 1-294), DJ region (nucleotides 294-342) and N-terminal sequence of a constant region (nucleotides 342-354)). The nucleotide sequences (and encoded amino acid sequences) are also provided in SEQ ID NOs: 37 and 38 (A387 light chain nucleotide and amino acid sequences, respectively), 39 and 40 (A387 heavy chain nucleotide and amino acid sequences, respectively), 41 and 42 (B436 light chain

- 232 -

nucleotide and amino acid sequences, respectively), 43 and 44 (B436 heavy chain nucleotide and amino acid sequences, respectively).

Results

Key: V-J regions are underlined. Regions determined by N-terminal amino acid sequencing are double-underlined. Regions not underlined are the N-terminal portions of the C regions.

A387 light (kappa) chain (SEQUENCE ID NO. 12)

	1	<u>DIVLTQSPAT</u>	<u>LSVSPGDSVS</u>	<u>LSCRASQIS</u>	<u>NNLHWYQKS</u>	<u>HESPRILIKY</u>
	51	<u>ASQSIYGIPS</u>	<u>RFSGSGSGTE</u>	<u>FTLIVNSVGT</u>	<u>EDEFGMYECQQ</u>	<u>SHSWPLTFGT</u>
10	101	<u>GTKLELKRAD</u>	<u>AAPTVSIFPP</u>	<u>SSEQLTSGGA</u>	<u>SVVCFLNIFY</u>	<u>PRDINVKWKI</u>
	151	DGSERQNGV				

A387 heavy (IgG_{2a}) chain (SEQUENCE ID NO.14)

	1	<u>EVKL</u> <u>VESGGD</u>	<u>LVKPGGSLKL</u>	<u>ACAASGFES</u>	<u>NDAMSWVRQT</u>	<u>PEKRELVVAS</u>
15	51	<u>ISSVGN</u> <u>TYYP</u>	<u>DSVKGR</u> <u>FETIS</u>	<u>RDNARNILYL</u>	<u>QMSSVR</u> <u>SEDT</u>	<u>AMYYCARGYG</u>
	101	<u>VSPWESYWGQ</u>	<u>GTLVTVSSAK</u>	TT		

B436 light (kappa) chain (SEQUENCE ID NO. 16)

	1	<u>DVI</u> <u>MTQTPLS</u>	<u>LPVSLGDQAS</u>	<u>ISCRSSQNTV</u>	<u>HSSGNTYLEW</u>	<u>YLQKPGQSPK</u>
20	51	<u>LLIYKVS</u> <u>NRF</u>	<u>SGVPDRESGS</u>	<u>GSGTDFTLKI</u>	<u>SRVEAEDLGI</u>	<u>YYCFQGSHVP</u>
	101	<u>YTEGGG</u> <u>TKLE</u>	<u>IKRADAAPT</u> <u>V</u>	<u>SIFPPS</u> <u>SEQL</u>	<u>TSGGASV</u> <u>VC</u> <u>F</u>	<u>LNNFYPRDIN</u>
	151	VKWKIDGSR	QNGV			

B436 heavy (IgG_{2a}) chain (SEQUENCE ID NO. 18)

25	1	<u>EVML</u> <u>VESGGG</u>	<u>LVKPGGSLKL</u>	<u>SCVASGFES</u>	<u>RYTMSWVRQT</u>	<u>PAKRELVVAT</u>
	51	<u>INEGNG</u> <u>NTYY</u>	<u>PDSVKG</u> <u>RETI</u>	<u>SRDNARNTLY</u>	<u>LQMSSL</u> <u>RSED</u>	<u>TAMYYCTSLN</u>
	101	<u>WAYWGQ</u> <u>GTLV</u>	<u>TVSSAKTT</u>			

EXAMPLE 5

30 A β 42 and A β 40 Sandwich ELISAs

Sandwich ELISAs (Enzyme-Linked Immunosorbent Assays) have been developed for specific detection of A β 42 and A β 40 peptides. An anti-A β 42 selective monoclonal antibody or anti-A β 40 selective monoclonal antibody (prepared to A β 30-40

- 233 -

- peptide using the same protocol as described for A β 42 antibody production) was coated on white microtiter 96-well plates (50 μ l at ~5 μ g/ml) in PBS, pH 7.4. Following overnight coating at 4 °C, wells were blocked with 200 μ l of 3% BSA, Fraction V (Sigma, St. Louis, MO) and incubated with A β peptides for 1 hour at room temperature.
- 5 Wells were washed three times with 200 μ l of PBS/0.1% Tween-20. After washing, wells were incubated with anti-A β 1-12 conjugated to alkaline phosphatase (~0.5 μ g/ml) for 1 hour. Wells were washed three times with 200 μ l of PBS/0.1% Tween-20 and CDP-Star chemiluminescence substrate (Tropix, Inc.) was added at 50 μ l/well and incubated for 15 min. The luminescence was then quantified on an ABI luminometer.
- 10 Results show a large linear range of 75-2000 pg/well, high dynamic range of 3-30 fold over background in linear range (signal:noise), low sensitivity limit <20 pg/well, and >1000-fold selectivity for A β 42 over other A β peptides, making the assay highly amenable to high throughput screening.

EXAMPLE 6

15 AB42/A β 40 high-throughput screening assay

- A selective A β 42/A β 40 high throughput 384-well screen to identify compounds that do not affect A β 40 levels has been developed. Due to the high sensitivity and selectivity of the A β 42/A β 40 ELISA, this assay was formatted for use in 384-well plates for high throughput screening for compounds that selectively decrease A β 42 levels while
- 20 not affecting A β 40 levels.

- Human neuroblastoma cells (SH-SY5Y) were obtained from ATCC (CRL-2266) and transfected with human APP₇₅₁ in a pcDNA.1 vector containing a neomycin resistant site. Cells were selected with 400 μ g/ml G418 (Gibco) and cloned by limiting dilution. Cells expressing the amyloid precursor protein (APP₇₅₁) were plated in 384-wells and
- 25 allowed to adhere for 24 hours. The cells were treated with a dose-response of DAPT (a positive control inhibitor used to inhibit A β 42 production) ranging from 1 nM to 1 μ M for 18 hours. Supernatant was then removed and assayed in the A β 42 ELISA. The ELISA was carried out by coating white microtiter 384-well plates with 25 μ l of ~5 μ g/ml solution of A β 42 selective monoclonal antibody (A387) in PBS. Following
- 30 overnight coating at 4 °C, wells were blocked with 50 μ l of 3% BSA/PBS, Fraction V

- 234 -

(Sigma, St. Louis, MO) and incubated with cell supernatant for 1 hour at room temperature. Plates were washed three times with 50 μ l of PBS/0.1% Tween-20. After washing, wells were incubated with 25 μ l of anti-A β 1-12 conjugated to alkaline phosphatase (\sim 0.5 μ g/ml) for 2 hours. Wells were washed three times with 50 μ l of PCS/0.1% Tween-20 and 25 μ l of CDP-Star chemiluminescence substrate (Tropix, Inc.) was added and incubated for 30 minutes at room temperature. Luminescence was quantified on an Analyst HT. The assay was repeated with a test library of compounds. Compound concentrations were \sim 30 μ M. 1 μ M DAPT was used as a positive control and DMSO vehicle alone (0.12%) was added as a negative control. The data showed acceptable signal to background (\sim 7-10 fold) with the positive control wells clearly distinguishable from the vehicle controls. Data from the test library screen showed that the hit criteria of $<$ 50% of plate median (50% inhibition)) is outside the normal distribution of the data therefore, compounds showing $>$ 50% inhibition in this primary screen were chosen for further follow-up assays such as A β 40 inhibition and cytotoxic assays. The % coefficient of variation range measured was 15-17%. Taken together, these data indicate that the assay (when performed in duplicate) has a $>$ 95% chance of identifying inhibitors.

Test compounds which show $>$ 50% inhibition for A β 42 levels are then tested for their effects on A β 40 levels using a similar assay except that the coat antibody is A β 40-specific. Furthermore, compounds are assessed for cytotoxicity using Alamar Blue (Biosource, Camarillo, CA) according to manufacturer's recommendations. Briefly, 10% Alamar Blue is added to cells after incubation of compound for 18h and incubated for 4 hours at room temperature, after which fluorescence is read on a spectrophotometer. Compounds that showed $>$ 40% cytotoxicity were eliminated as hits. The screening methods have also been performed using CHO cells containing DNA that encodes human APP₆₉₅ and human PS1. The screening methods may also be performed using mouse neuroblastoma (N2a) cells expressing APP. N2a cells can be transfected with DNA encoding APP as described in Example 8.

EXAMPLE 7

30 Analysis of Processing of LRP

- 235 -

Endogenous LRP protein of N2a cells expressing human wild-type and mutant PS1 was analyzed and compared. Notch and APP protein in the cells was also analyzed as a reference for PS1-dependent protein cleavage.

Stable recombinant N2a cells that had been transfected with DNA encoding wild-type human APP695 (see, *e.g.*, SEQ ID NO: 30 and GenBank Accession no. Y00264) and DNA encoding either wild-type human PS1 (see, *e.g.*, SEQ ID NO: 5) or mutant human PS1 were grown overnight to near 70% confluence in a 10-cm tissue-culture dish. Two mutant PS1 cell lines were used: Δ 1,2 and D385A. The Δ 1,2 cell line expresses defective (*i.e.*, loss of function) PS1 proteins encoded by nucleic acid lacking exons 1 and 2 of the human PS1 gene. The D385A cell line contains nucleic acid coding for an alanine instead of an aspartic acid residue at amino acid 385 (see, *e.g.*, SEQ ID NO: 6 for amino acid sequence of a wild-type human PS1) which is essential to PS1 function.

The Δ 1,2 cells and the D385A cells were also transiently transfected with 2 μ g of DNA encoding an amino-terminal truncated form of human NOTCH Δ E containing residues 1-26 (signal sequence; see, *e.g.*, SEQ ID NO: 31) and residues 1718-2195 (see, *e.g.*, SEQ ID NO: 31) with the methionine 1738 mutated to valine to prevent alternative translation initiation at that site. The DNA construct also contains nucleic acid sequence encoding a carboxy-terminal V5 antibody epitope which is comprised of the 14-amino acid sequence; Gly-Lys-Pro-Ile-Pro-Asn-Pro-Leu-Leu-Gly-Leu-Asp-Ser-Thr added to the carboxy-terminal end of the Notch amino acid sequence so that a V5 antibody could be used to detect the Notch Δ E or the NICD. This construct encodes a ~55-60 kDa protein. Transfection was carried out using Quiagen's effectene reagent for 20 h. Cells were then plated at 1.2×10^6 cells/well in 6-well plates. After 28 h, cells were treated +/- DAPT (1 μ M) and then lysed in 200 μ M lysate buffer (10% 10X TBS, 0.05% Tween 20, 1% Triton X-100, and a protease inhibitor cocktail) after 19 h of treatment. Cells were centrifuged at 10,000 rpm for 5 min and the supernatant was removed. The supernatant of the lysates was then separated on 8% Tris-Glycine gels and proteins were transferred to nitrocellulose membrane. The membranes then were blocked for an hour with 10% nonfat dry milk and probed with the anti-V5 (1:2000) primary antibody (Invitrogen, San Diego) to detect accumulation of the Notch substrate, anti-LRP antibody R9377 (as

- 236 -

described in Example 3) to probe for LRP CTFs, and anti-APP antibody R8666 (a rabbit antibody prepared to the carboxy-terminal region; amino acids C-EVPTYKFFEQMQN conjugated to ovalbumin through the amino-terminal cysteine residue) to visualize APP CTFs. Bound antibody was detected using the ECL SuperSignal system (Pierce) after
5 incubation with anti-rabbit horseradish peroxidase-coupled secondary antibodies (Sigma). Samples were assayed in duplicate.

In lysates of the wild-type PS1 cells, an approximately 20 kDa protein fragment was observed in the presence of the PS1 inhibitor DAPT. The fragment is one that is recognized and bound by the polyclonal antibody R9377 generated against a carboxyl-
10 terminal peptide (the carboxyl-terminal 13 amino acids) of human LRP (C-GRGPEDEIGDPLA) and thus is one derived from a C-terminal portion of LRP. Accumulation of this fragment was not detected in lysates of wild-type PS1 cells not treated with DAPT. Because little to no protein is detected in DAPT-treated cell lysates by the R9377 antibody generated against a C-terminal peptide of LRP, but a peptide
15 fragment is detected at significant levels by the antibody in lysates of DAPT-treated cells, it can be concluded that a PS1-dependent activity cleaves LRP in such a way as to eliminate the epitope sequence on LRP that is recognized by antibody R9377. These results are consistent with presenilin-dependent cleavage of LRP.

Similar results were obtained in the analyses of lysates of wild-type PS1 cells
20 using antibodies reactive with APP and Notch, respectively. In lysates of wild-type PS1 cells treated with DAPT, two peptide fragments (~17 kDa and ~14 kDa representing the C99 and C83, β - and α -secretase cleavage products, respectively) were readily detected by the anti-APP antibody R8666. In lysates of cells that were not treated with DAPT, little to no protein was detected by the R8666 antibody. In lysates of wild-type PS1 cells
25 treated with DAPT, one peptide fragment was detected by the anti-V5 antibody. Although this fragment was also detected in lysates of wild-type PS1 cells that were not treated with DAPT, the amount of the fragment detected in the lysates of the DAPT-treated cells was significantly greater than in the lysates of the untreated cells.

The results of the analyses of lysates of DAPT-treated and untreated wild-type
30 PS1 cells using anti-APP and anti-Notch-V5 fusion protein antibodies are consistent with

- 237 -

inhibition and non-inhibition, respectively, of the PS1-dependent cleavage of these presenilin substrates (i.e., APP and Notch) at a site in a C-terminal portion of these proteins. Thus, the similar findings in the analysis of LRP protein in the cell lysates and the APP and Notch analyses supports the conclusion of a presenilin-dependent cleavage
5 of LRP.

In the lysates of the DAPT-treated and untreated $\Delta 1,2$ mutant cell line, the ~20 kDa LRP CTF was equally evident at significant levels. Similarly, the same fragments were detected in the lysates of the DAPT-treated and untreated $\Delta 1,2$ mutant cells by the R8666 antibody, and the anti-Notch-V5 fusion protein antibody also detected the same
10 fragment in lysates of the treated and untreated mutant cells. The same results were obtained in analyses of the lysates of the DAPT-treated and untreated mutant D385A cells with the antibodies for the detection of LRP, APP and Notch peptides. These results obtained with cells that do not express functional PS1 provide confirmation that the results observed with DAPT-treated and untreated wild-type PS1 cell lysates are due
15 to the inhibition and non-inhibition of a PS1-dependent activity.

Furthermore, a comparison of the very minimal levels of the peptide fragments detected in immunoassays of lysates of wild-type PS1 cells that were not treated with DAPT with the significant levels of the peptide fragments detected in lysates of D385A mutant cells, indicated an approximate 40-60% loss of PS1 activity in the mutant cells
20 relative to wild-type PS1 cells. Because accumulation of the ~20 kDa LRP fragment in the presence of DAPT and in PS1 mutant cell lines parallels the accumulation of the APP and Notch fragments, these results indicate that LRP undergoes a PS1-dependent cleavage.

EXAMPLE 8

25 Presenilin/ γ -secretase Assays

N2a mouse neuroblastoma cells (ATCC, Rockville, MD) transfected with APP_{WT} (Acc. No. Y00264) were incubated with DAPT (1 μ M or 1 mM) or vehicle control (DMSO) for 24 hours. Lysates were then prepared by first washing the cell layer three times with isotonic PBS. To each 96-well, 50 μ l of lysate buffer (TBS, 1% Triton X-
30 100, 5 mM EDTA, 0.2% Tween-20, 10 μ M leupeptin, 1 mM PMSF) was added and cells

- 238 -

were removed by agitating with a pipet tip. Cell lysates were spun for 5 min at 10,000 rpm in a microfuge and the supernatant was collected.

The lysates were then separated on 4-20% Novex gels and probed by immunoblotting with the anti-LRP polyclonal antibody (R9377). Results showed
5 accumulation of a 20 kDa protein in lysates of cells that had been treated with DAPT. This band represented a carboxyl-terminal fragment of LRP. This accumulation of LRP CTFs paralleled the accumulation of APP CTFs, a finding that demonstrates that LRP is a distinct presenilin substrate and can be used to quantitate presenilin activity. The LRP assay can be used to profile test compounds that modulate A β levels, and, in particular
10 A β 42 levels (such as can be identified in the high-throughput assay; see EXAMPLE 6), with respect to possible effects on presenilin activity. In one aspect, compounds that are identified as agents that reduce A β 42 levels (*e.g.*, by $\geq 50\%$ at, *e.g.*, 30 μ M; see EXAMPLE 6) are tested for any effects on presenilin activity in the LRP assay in order to identify A β 42-reducing compounds that have minimal to no inhibitory activity with
15 respect to presenilin-dependent LRP processing activity. Compounds were chosen that had $<20\%$ increase (at the highest tested concentration of 30 μ M) of LRP-CTFs as compared to the DAPT positive control.

EXAMPLE 9

20

Characterization of binding properties of A β antibodies

(1) Assessment of binding to different forms of A β

(a) Non-reducing gel electrophoresis and immunoblotting

25 **Methods.** A β 40 or A β 42 peptide standards (Bachem) (250 ng in 10 μ l) were mixed with 10 μ l native sample buffer (Invitrogen). Samples were run on 18% Novex 10-well gels at constant voltage (150 V) using native sample buffer (Invitrogen). Novex rainbow standards (250 kd to 4 kd) were used as molecular weight controls. When the dye front reached the bottom of the gel, proteins were transferred to 0.45 micron PVDF
30 filters (pre-wetted in methanol) at 100 mA constant current in 1X CAPS buffer (10 mM

- 239 -

CAPS), 10% methanol, pH 11.0 for 90 min. Filters were blocked with TBS, 10% dry milk, pH 7.4 for 60 min at room temperature. Filters were then incubated overnight at 4°C in a solution of TBS, 3% dry milk, 0.1% Tween-20 containing primary antibody (1-5 μ g/ml of A387 or B436 conjugated to biotin), followed by three five-minute washes in TBS containing 0.1% Tween-20. After washing, filters were incubated in anti-biotin peroxidase-conjugated secondary antibody (Sigma; 1:2000 in TBS, 3% dry milk, 0.1% Tween-20) for 2 h at room temperature, and washed six times for five minutes each with TBS, 0.1% Tween-20. Signals were detected with the Chemiluminescence Supersignal ECL system (Pierce).

10

Results. By non-denaturing gel electrophoresis and immunoblotting as described above, A β 42 peptides run at positions consistent with various forms, including insoluble fibrils (near top of the gel), high molecular weight oligomers (> ~100 kd) and low molecular weight oligomers such as pentamers (~20 kd) and dimers (~10 kd). A β 40 peptides run at positions consistent with insoluble fibrillar and low molecular weight oligomeric forms. B436 antibody was shown to detect all forms of both A β 40 and A β 42 peptides. As expected, A387 did not recognize any forms of A β 40. A387 antibody did not significantly recognize either A β 42 fibrils or high molecular weight oligomers, and instead primarily recognized A β 42 low molecular weight oligomers.

20

(b) ELISA assays in presence and absence of bathocuprine

Methods. Dynex Microfluor-2 White Flat-bottom 96-well plates were coated overnight with 2-10 μ g/well of A387 antibody. A β 42 peptide standard (Bachem) was added at concentrations ranging from 5000 pg/well to 0.08 pg/well at half log intervals, in either DMEM complete medium or DMEM complete medium containing 2 mM of the metal chelator bathocuprine, and plates incubated at room temperature for 2 h. After washing in PBS/0.1% Tween-20, alkaline phosphatase-labeled B436 antibody (~0.5 μ g/ml) in 1% BSA/TBS/0.1% Tween-20 was added, and plates incubated at room temperature for 2 h. After washing, CDP-Star-Sapphire Luminescence Substrate

30

- 240 -

(Applied Biosystems) was added and the plates incubated for 5-15 min in the dark. Signal was then detected using an ABI TR717 Luminometer.

Results. Bathocuprine has been shown to solubilize A β aggregates into low molecular weight oligomers (Cherny et al. (1999) *J. Biol. Chem.* 274:23223-23228). In the presence of bathocuprine, A387 bound more A β 42 peptide than in the absence of bathocuprine. These results are consistent with A387 recognizing lower molecular weight oligomers of A β 42 peptide.

10 (2) Assessment of antibody binding to A β in plasma

Methods. Mouse blood was obtained by cardiac puncture at sacrifice. Briefly, mice were sedated by standard anesthesia. Upon sedation, each mouse was placed in dorsal recumbence and a 26-gauge needle attached to a heparinized 1 cc syringe inserted into the thorax through the diaphragm to an approximate depth of 2 cm. Light suction was applied to the needle and placement in the cardiac (ventricular) chamber of the mouse confirmed by blood flow to the syringe chamber. Blood was aspirated until flow ceased. To obtain plasma, blood samples from each mouse were spun at 3,000 RPM for 10 minutes, and the supernatant collected. Plasma was then frozen until analyzed.

20 For the immunoprecipitation assay, mouse plasma was centrifuged at low speed for 5 min to remove precipitated material, and the supernatant diluted in PBS 1:2. Human A β 40 and A β 42 peptide standards (Bachem) were added at 300 ng/ml to the diluted plasma, and the samples incubated with 1 ml of Sepharose beads for 1 h at 4°C. Beads were precipitated, and samples divided into 1 ml aliquots. To 1 ml of sample, 25 biotin-labeled B436 or A387 antibodies (~10 μ g/ml) were added, together with 40 μ l of Streptavidin:Sepharose beads (Pierce), and the samples rocked overnight at 4°C. Samples were spun to pellet the beads, which were washed twice with 1 ml PBS-0.1% Tween-20. 30 μ l of NuPAGE sample buffer (Invitrogen) was added, samples were boiled for 3 min, and supernatants loaded onto a 10% Bis-Tris NuPAGE gel (Invitrogen) 30 at 125V. Molecular weight standards from 185 kDa to 3 kDa were also run. After

- 241 -

electrophoresis, proteins were transferred to PVDF filters at 100 mA for 90 min. Filters were then blocked in TBS-Tween containing 5% non-fat dry milk for 2 h. Blocked filters were incubated overnight with biotin-labeled 6E10 antibody, which recognizes the N-terminus of A β (1:500; Signet Laboratories), and then with HRP-labeled anti-HRP for
5 1 h (1:2000; Sigma). Signal was detected following incubation with Super Signal (Pierce Chemical Co.) for 1 min.

Results. Both A387 and B436 antibodies were able to immunoprecipitate human A β 42 spiked into mouse plasma. The detected A β 42 had an apparent molecular weight
10 consistent with monomeric form of the peptide.

(3) Assessment of binding to A β in brain

Methods. The animals used in these experiments were either C57 mice, or
15 Tg2576 mice of three or six months of age. Tg2576 mice express human APP695 with the Swedish (Lys670Asn, Met671Leu) double mutation under the control of the hamster prion protein gene promoter (Hsiao et al. (1996) Science 274:99-102; U.S. Patent No. 5,877,399). Mouse brain samples were prepared at sacrifice by brain removal and knife bisection along the superior sagittal sulcus from the cortical surface to the extreme ventral
20 surface. One brain hemi-section from each animal was snap frozen in liquid nitrogen. Frozen brain hemi-sections were weighed and transferred to thick-walled polyallomer centrifuge tubes. A 10x volume (wt:vol) of 70% formic acid was added to each sample. The samples were briefly homogenized over ice, then centrifuged @ 100,000 x g for 1 hr at 4° C. The clear supernatant between the lipid layer and pellet was collected and its
25 volume determined. An 11x volume (vol:vol) 1M Tris Base was added to neutralize the sample to pH range ~ 8 – 8.5, and aliquots were frozen at –80° C until analyzed.

For A387 ELISA analysis of brain samples, A387 antibody was coated onto plates, and the ELISA assay performed essentially as described in Section (1)(b) of this Example. ELISA analysis was also performed using the Human Beta-Amyloid (Abeta)
30 [1-42] Fluorometric ELISA Kit (Biosource, catalog # 88-344), following manufacturers'

- 242 -

directions. The coat antibody in the Biosource Kit is a monoclonal antibody directed against the N-terminus of human A β . The detection antibody is a rabbit polyclonal antibody that recognizes human A β 42, but not human A β 40 or mouse A β . The rabbit antibody is detected using an anti-rabbit IgG-alkaline phosphatase conjugate and a
5 fluorescent substrate. For both the Biosource and the A387 ELISA assays, A β 42 standard curves were prepared from serial dilutions of A β 42 peptide (Bachem; stored in hexafluoroisopropanol) in C57 brain homogenate.

Results. Using the Biosource Kit ELISA assay, the amount of formic acid-
10 extractable A β 42 detected in brains of Tg2576 animals was not significantly different from background (C57 brains). However, using A387 antibody and the ELISA protocol described herein, the amount of formic acid-extractable A β 42 detected in brains of Tg2576 animals was about three-fold higher than background in the linear range of the A β 42 standard curve.

15

EXAMPLE 10

**Method for administering A β monoclonal antibodies to animals and for assessing
20 the effects of the antibodies on A β levels and amyloid plaques**

Animals. TASD41 transgenic mice, which express human APP751 cDNA containing the London (V717I) and Swedish (K670M/N671L) mutations under the control of the murine Thy-1 gene, are used. The generation and properties of these
25 animals (line 41) are described in Rockenstein *et al.* (2001) *J. Neurosci. Res.* 66:573-582. Briefly, Rockenstein *et al.* showed that TASD41 mice exhibit mature plaques in the frontal cortex as early as 3-4 months of age, and by 5-7 months also exhibit plaques in the hippocampus, thalamus and olfactory region. By ultrastructural and double immunostaining analysis, these plaques were shown to contain dystrophic neuritis
30 immunoreactive with antibodies against APP, synaptophysin, neurofilament and tau.

- 243 -

As such, the TASD41 mouse is a useful animal model of Alzheimer's disease.

Administration of antibodies. TASD41 mice of either about 4 months of age, or about 8 months of age, are divided into groups of 6-8 age-matched animals. Once a
5 week for 3-6 months, each animal receives an intraperitoneal injection of either 500 μ g of A387 antibody in saline, 500 μ g of B436 antibody in saline, or 500 μ g of control IgG in saline, according to the group.

A β ELISA assays. At sacrifice, plasma and brain samples are prepared from each
10 animal as described in Example 9, and ELISA assays performed according to the procedure described in Example 9. A difference in A β 40 or A β 42 levels, or of particular A β 40 or A β 42 forms, can be detected.

Histopathology. One hemi-brain from each animal is fixed by immersion in 4%
15 paraformaldehyde in PBS (pH 7.4). A series of consecutive 40 μ M sagittal sections are cut using a Leica Vibratome and stored in cryoprotectant solution at -20°C. Sections are stained with Thioflavine S (which binds amyloid plaques) and with Cresyl Violet, and analyzed under fluorescent and bright field microscopy, respectively. A difference in abundance of amyloid plaques between antibody-treated and Ig-treated animals can be
20 observed.

Since modifications will be apparent to those of skill in this art, it is intended that this invention be limited only by the scope of the appended claims.

25

- 244 -

What is claimed:

1. A polypeptide, comprising a sequence of amino acids that is selectively reactive with A β 42 and preferentially binds to low molecular weight forms of A β 42.
- 5 2. The polypeptide of claim 1, comprising at least one complementarity-determining region (CDR) selected from the group consisting of CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2 or CDR-H3 of antibody A387.
3. The polypeptide of claim 1, comprising CDR-L1, CDR-L2, CDR-L3,
10 CDR-H1, CDR-H2 and CDR-H3 of antibody A387.
4. The polypeptide of claim 1, wherein at least one CDR is selected from the group consisting of amino acids 24-34 of SEQ ID NO:12, amino acids 50-56 of SEQ ID NO:12, amino acids 89-97 of SEQ ID NO:12, amino acids 26-35 of SEQ ID NO:14,
15 amino acids 31-35 of SEQ ID NO:14, amino acids 26-31 of SEQ ID NO:14, amino acids 50-65 of SEQ ID NO:14, amino acids 50-58 of SEQ ID NO:14, and amino acids 98-107 of SEQ ID NO:14.
5. The polypeptide of claim 1, comprising at least a portion of a variable
20 domain of the light chain or the heavy chain of an A β antibody.
6. The polypeptide 5, wherein the variable domain is selected from the group consisting of the light chain variable domain of A387, the heavy chain variable domain of A387, a polypeptide with at least 85% identity to the light chain variable domain of
25 A387; a polypeptide with at least 85% identity to the heavy chain variable domain of A387.
7. The polypeptide of claim 1, further comprising a scaffold.
- 30 8. The polypeptide of claim 7, wherein the scaffold is a polypeptide scaffold.

- 245 -

9. The polypeptide of claim 7, wherein the scaffold is a human polypeptide scaffold.
- 5 10. The polypeptide of claim 7, wherein the scaffold is an antibody scaffold.
11. The polypeptide of claim 10, wherein the antibody scaffold is selected from the group consisting of an Fv fragment scaffold, an Fab fragment scaffold, and a single-chain (scFv) fragment.
- 10 12. The polypeptide of claim 1, further comprising a detectable moiety.
13. The polypeptide of claim 1, further comprising a clearance domain.
- 15 14. The polypeptide of claim 13, wherein the clearance domain is a ligand for an Fc receptor.
- 15 15. A polypeptide, comprising at least one complementarity-determining region (CDR) selected from the group consisting of CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2 or CDR-H3 of antibody A387.
- 20 16. The polypeptide of claim 15 comprising CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2 and CDR-H3 of antibody A387.
- 25 17. The polypeptide of claim 15, wherein at least one CDR is selected from the group consisting of amino acids 24-34 of SEQ ID NO:12, amino acids 50-56 of SEQ ID NO:12, amino acids 89-97 of SEQ ID NO:12, amino acids 26-35 of SEQ ID NO:14, amino acids 31-35 of SEQ ID NO:14, amino acids 26-31 of SEQ ID NO:14, amino acids 50-65 of SEQ ID NO:14, amino acids 50-58 of SEQ ID NO:14, and amino acids 98-107
- 30 of SEQ ID NO:14.

- 246 -

18. The polypeptide of any of claims 15-17 further comprising a scaffold.
19. The polypeptide of claim 18 wherein the scaffold comprises a solid
5 support.
20. The polypeptide of claim 18 wherein the scaffold is a polypeptide scaffold.
- 10 21. The polypeptide of claim 18, wherein the scaffold is a human polypeptide scaffold.
22. The polypeptide of claim 18, wherein the scaffold is an antibody scaffold.
- 15 23. The polypeptide of claim 22, wherein the antibody scaffold is selected from the group consisting of an Fv fragment scaffold, an Fab fragment scaffold, and a single-chain (scFv) fragment.
- 20 24. The polypeptide of any of claims 15-17, wherein the polypeptide is a chimeric polypeptide.
25. The polypeptide of any of claims 15-17, wherein the polypeptide is an antibody.
- 25 26.. The polypeptide of any of claims 15-17, further comprising a clearance domain.
27. The polypeptide of claim 26, wherein the clearance domain is a ligand for
30 an Fc receptor.

- 247 -

28. The polypeptide of any of claims 15-17, further comprising a detectable moiety.
- 5 29. The polypeptide of claim 16, which comprises amino acids 1-95 of SEQ ID NO:12, or a fragment thereof and/or comprises amino acids 1-97 of SEQ ID NO:14, or a fragment thereof.
- 10 30. The polypeptide of claim 29, further comprising one or more joining regions.
- 15 31. The polypeptide of claim 30, wherein at least one joining region comprises amino acids 96-107 of SEQ ID NO:12 or amino acids 98-118 of SEQ ID NO:14.
32. The polypeptide of claim 30, wherein at least one joining region comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 46, 48, 50, 52, 54, 55, 57, 59, 61, 67, 73, 75, 77, 79, 89 and 91.
- 20 33. The polypeptide of claim 29, further comprising one or more constant regions.
- 25 34. The polypeptide of claim 33, wherein the constant region is a mouse constant region.
35. The polypeptide of claim 34, wherein the constant region comprises an amino acid sequence selected from the group consisting of SEQ ID NOS:63, 65, 69 and 71.
- 30 36. The polypeptide of claim 33, wherein the constant region is a human

- 248 -

constant region.

37. The polypeptide of claim 36, wherein the constant region comprises an amino acid sequence selected from the group consisting of SEQ ID NOS:81, 83, 85 and
5 87.

38. The polypeptide of claim 16 comprising the amino acid sequence of SEQ ID NO:97 and/or SEQ ID NO:98.

10 39. The polypeptide of any of claims 15-17, which is specifically reactive with at least one A□

40. The polypeptide of claim 39, wherein A□ is A□□□

15 41. The polypeptide of claim 39, which binds A□□□ without substantially binding other A□.

42. A polypeptide comprising at least one complementarity-determining region (CDR) selected from the group consisting of CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2 or CDR-H3 of antibody B436.
20

43. The polypeptide of claim 42, comprising CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2 and CDR-H3 of antibody B436.

25 44. The polypeptide of claim 43, wherein at least one CDR is selected from the group consisting of amino acids 24-39 of SEQ ID NO:16, amino acids 55-61 of SEQ ID NO:16, amino acids 94-102 of SEQ ID NO:16, amino acids 26-35 of SEQ ID NO:18, amino acids 31-35 of SEQ ID NO:18, amino acids 26-31 of SEQ ID NO:18, amino acids 50-66 of SEQ ID NO:18, amino acids 50-59 of SEQ ID NO:18, and amino acids 99-103
30 of SEQ ID NO:18.

- 249 -

45. The polypeptide of any of claims 42-44 further comprising a scaffold.
- 5 46. The polypeptide of any of claims 45, wherein the scaffold comprises a solid support.
47. The polypeptide of any of claims 45, wherein the scaffold is a polypeptide scaffold.
- 10 48. The polypeptide of claim 45, wherein the scaffold is a human polypeptide scaffold.
49. The polypeptide of claim 45, wherein the scaffold is an antibody scaffold.
- 15 50. The polypeptide of claim 49, wherein the antibody scaffold is selected from the group consisting of an Fv fragment scaffold, an Fab fragment scaffold, and a single-chain (scFv) fragment.
- 20 51. The polypeptide of any of claims 42-44, wherein the polypeptide is a chimeric polypeptide.
52. The polypeptide of any of claims 42-44, wherein the polypeptide is an antibody.
- 25 53. The polypeptide of any of claims 42-44, further comprising a clearance domain.
54. The polypeptide of claim 53, wherein the clearance domain is a ligand for
30 an Fc receptor.

- 250 -

55. The polypeptide of any of claims 42-44, further comprising a detectable moiety.
- 5 56. The polypeptide of claim 43, which comprises amino acids 1-100 of SEQ ID NO:16, or a fragment thereof and/or comprises amino acids 1-98 of SEQ ID NO:18, or a fragment thereof.
- 10 57. The polypeptide of claim 56, further comprising one or more joining regions.
58. The polypeptide of claim 57 wherein at least one joining region comprises amino acids 101-112 of SEQ ID NO:16 or amino acids 99-114 of SEQ ID NO:18.
- 15 59. The polypeptide of claim 57, wherein at least one joining region comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 46, 48, 50, 52, 54, 55, 57, 59, 61, 67, 73, 75, 77, 79, 89 and 91.
- 20 60. The polypeptide of claim 56, further comprising one or more constant regions.
61. The polypeptide of claim 60, wherein the constant region is a mouse constant region.
- 25 62. The polypeptide of claim 61, wherein the constant region comprises an amino acid sequence selected from the group consisting of SEQ ID NOS:63, 65, 69 and 71.
- 30 63. The polypeptide of claim 60, wherein the constant region is a human constant region.

- 251 -

64. The polypeptide of claim 63, wherein the constant region comprises an amino acid sequence selected from the group consisting of SEQ ID NOS:81, 83, 85 and 87.

5

65. The polypeptide of claim 43, comprising the amino acid sequence of SEQ ID NO:99 and/or SEQ ID NO:100.

66. The polypeptide of any of claims 42-44 which is specifically reactive with at least one A β peptide.

10

67. A nucleic acid molecule encoding the polypeptide of any of claims 1-41.

68. A nucleic acid molecule encoding the polypeptide of any of claims 42-66.

15

69. A kit, comprising the polypeptide of any of claims 1-41.

70. A kit, comprising the polypeptide of any of claims 42-66.

71. A method for assessing the presence or amount of A \square in a sample, comprising:

20

contacting the polypeptide of any of claims 1-14, 39-41 or 66 with the sample under conditions whereby a complex is formed between the polypeptide and A \square , and assessing the presence or amount of the complex in the sample, and thereby determining the presence or amount of A \square in the sample.

25

72. The method of claim 71, wherein the sample is selected from the group consisting of a cell extract, extracellular medium, plasma, cerebrospinal fluid and brain.

73. The method of claim 71, wherein the presence or amount of the complex

30

- 252 -

is assessed using an enzyme-linked immunosorbent assay (ELISA).

74. A method, comprising administering to a subject the polypeptide of any of claims 1-66.

5

75. A method of binding A β comprising administering to a subject the polypeptide of any of claims 1-14, 39-41 or 66 to bind A β .

76. The method of claim 74 or 75, wherein the subject has, or is at risk of developing, a disease associated with accumulation of A β .

10

77. The method of claim 76, wherein the disease is Alzheimer's disease.

78. A method of reducing A β level in an subject, comprising administering to the subject an effective amount of the polypeptide of any of claims 1-14, 39-41 or 66 to reduce the level of at least one A β peptide.

15

79. The method of claim 78, wherein the subject has, or is at risk of developing, a disease associated with accumulation of A β .

20

80. The method of claim 79, wherein the disease is Alzheimer's disease.

81. The method of claim 78, wherein the level of at least one A β peptide in blood or plasma is reduced.

25

82. The method of claim 78, wherein the level of at least one A β peptide in brain is reduced.

83. A method for assessing presenilin activity, comprising:
contacting a sample containing a presenilin and/or fragment(s) thereof

30

- 253 -

with a lipoprotein receptor-related protein (LRP) and/or fragment(s) thereof; and
assessing the processing and/or cleavage of the LRP or fragment(s)
thereof.

- 5 84. A method for identifying an agent that modulates presenilin activity,
 comprising:
 contacting a sample containing a presenilin, and/or fragment(s) thereof,
 and a lipoprotein receptor-related protein (LRP), and/or fragment(s) thereof with a test
 agent; and
10 identifying an agent that alters the processing and/or cleavage of LRP
 and/or fragment(s) thereof.

85. A method for identifying a candidate agent for treatment or prophylaxis of
 a disease associated with an altered presenilin, comprising:
15 contacting a sample that contains an altered presenilin and/or fragment(s)
 thereof and a lipoprotein receptor-related protein (LRP) and/or fragment(s) thereof with a
 test agent, wherein the altered presenilin and/or fragment(s) thereof is associated with an
 altered cleavage and/or processing of LRP and/or fragment(s) thereof; and
 identifying a candidate agent that restores LRP cleavage and/or processing
20 to substantially that which occurs in the presence of a presenilin and/or fragment(s)
 thereof that is not associated with an altered cleavage and/or processing of LRP and/or
 fragment(s) thereof.

86. A method for modulating LRP, comprising altering the structure, function
25 and/or activity of a presenilin, and/or fragment(s) thereof, in a sample comprising LRP,
 and/or fragment(s) thereof, and a presenilin, and/or fragment(s) thereof, whereby the LRP
 is modulated.

87. A method for modulating LRP, comprising contacting a sample
30 comprising an LRP, and/or fragment(s) thereof, and presenilin, and/or fragment(s)

- 254 -

thereof, with an agent that modulates the presenilin and/or fragment(s) thereof or a presenilin-dependent activity, whereby LRP is modulated.

5 88. A method for identifying an agent that modulates A β levels, comprising:
 comparing the levels of bound A β binding protein in a test sample
 contacted with the test agent and a control sample not contacted with the test agent; and
 identifying an agent as an agent that modulates A β levels if the levels of
 bound A β binding protein differ in the test and control samples; wherein
 the sample comprises APP or portion(s) thereof; and
10 the A β binding protein comprises the polypeptide of any of claims 1-14,
 39-41 or 66.

 89. A method for identifying an agent that modulates A β 42 levels,
 comprising:
15 comparing the levels of bound A β binding protein in a test sample
 contacted with the test agent and a control sample not contacted with the test agent; and
 identifying an agent as an agent that modulates A β 42 levels if the levels of
 bound A β binding protein differ in the test and control samples; wherein
 the sample comprises APP or portion(s) thereof; and
20 the A β binding protein comprises a sequence of amino acids selected from
 the group consisting of amino acids 1-50, 1-60, 1-70, 1-80, 1-90, 1-91, 1-92, 1-93, 1-94,
 1-95, 1-96 and 1-97 of SEQ ID NO: 12 and 1-50, 1-60, 1-70, 1-80, 1-90, 1-91, 1-92, 1-
 93, 1-94, 1-95, 1-96 and 1-97 of SEQ ID NO: 14 and any amino acid sequences
 containing modifications of these amino acid sequences that retain the A β binding
25 properties of an antibody comprising one or both of the amino acid sequences set forth as
 amino acids 1-95 of SEQ ID NO: 12 and amino acids 1-97 of SEQ ID NO: 14.

 90. A method for identifying an agent that modulates A β levels, comprising:
 assessing a test agent that modulates A β 42 levels to determine if it
30 modulates the level of one or more other A β peptides; and

- 255 -

identifying an agent that modulates A β 42 levels to a greater extent than it modulates the level of one or more other A β peptides.

- 5 91. A method for identifying an agent that modulates A β levels, comprising:
 assessing a test agent that modulates A β 42 levels to determine if it
modulates the level of one or more other A β peptides; and
 identifying an agent that modulates A β 42 levels and A β 39 levels.
- 10 92. A method for identifying an agent that modulates A β levels, comprising:
 assessing a test agent that alters the cleavage of APP that produces one or
more A β peptides, the processing of APP, the processing of A β and/or the level of one or
more A β peptides to determine if it effects one or more presenilin-dependent activities
other than the presenilin-dependent processing of APP or portion(s) thereof; and
 identifying an agent that modulates A β levels without substantially
15 altering one or more presenilin-dependent activities other than the presenilin-dependent
processing of APP.
- 20 93. A method for identifying an agent that modulates A β levels, comprising:
 assessing a test agent that modulates the cleavage of APP that produces
one or more A β peptides, the processing of APP, the processing of A β and/or the level of
one or more A β peptides to determine if it effects the cleavage and/or processing of a
presenilin substrate and/or portion(s) thereof other than APP; and
 identifying an agent that modulates A β levels without substantially
altering the cleavage and/or processing of the presenilin substrate and/or portion(s)
25 thereof that is other than APP.
- 30 94. A method for identifying an agent that modulates A β levels, comprising:
 assessing a test agent that modulates the cleavage of APP that produces
one or more A β peptides, the processing of APP, the processing of A β and/or the level of
one or more A β peptides to determine if it effects the cleavage and/or processing of LRP

- 256 -

and/or portion(s) thereof; and

identifying an agent that modulates $A\beta$ levels without substantially altering the cleavage and/or processing of LRP and/or portion(s) thereof.

- 5 95. A system for use in assessing presenilin activity, comprising:
a source of presenilin activity;
a source of LRP (and/or portion(s) thereof); and
a reagent for determining LRP protein composition.
- 10 96. A kit comprising:
a reagent for assessing cleavage of APP that produces one or more $A\beta$
peptides, APP processing, $A\beta$ processing and/or $A\beta$ levels; and
a reagent for assessing cleavage and/or processing of a presenilin
substrate.
- 15 97 A method for identifying a candidate agent for the treatment or
prophylaxis of a disease, comprising:
contacting a sample that contains an altered test protein, and/or portion(s)
thereof, and APP, and/or portion(s) thereof, with a test agent, wherein the altered protein
20 is associated with altered $A\beta$ 42 production, catabolism, processing and/or $A\beta$ 42 levels;
and
identifying a candidate agent that restores $A\beta$ production, catabolism,
processing and/or $A\beta$ levels to substantially that which occurs in the presence of a test
protein and/or portion(s) thereof that is not associated with altered $A\beta$ 42 production,
25 catabolism, processing and/or $A\beta$ 42 levels without substantially altering the level of one
or more other $A\beta$ peptides.
98. A method for identifying a candidate agent for the treatment or
prophylaxis of a disease, comprising:
30 contacting a sample that contains an altered test protein, and/or portion(s)

- 257 -

thereof, and APP, and/or portion(s) thereof, with a test agent, wherein the altered protein is associated with altered A β production, catabolism, processing and/or A β levels; and identifying a candidate agent that restores A β production, catabolism, processing and/or A β levels to substantially that which occurs in the presence of a test protein and/or portion(s) thereof that is not associated with altered A β production, catabolism, processing and/or A β levels without substantially altering (a) one or more presenilin-dependent activities other than the presenilin-dependent processing of APP, (b) the cleavage and/or processing of a presenilin substrate and/or portion(s) thereof that is other than APP and/or (c) the cleavage and/or processing of LRP and/or portion(s) thereof.

99. A method for identifying an agent that modulates A β levels, comprising: assessing a test agent that alters the cleavage of APP that produces one or more A β peptides, the processing of APP, the processing of A β and/or the level of one or more A β peptides to determine if it affects one or more presenilin-dependent activities other than the presenilin-dependent processing of APP or portion(s) thereof that produces one or more A β peptides; and identifying an agent that modulates A β levels without substantially altering one or more presenilin-dependent activities other than the presenilin-dependent processing of APP or portion(s) thereof that produces one or more A β peptides.

100. A method for identifying an agent that modulates A β levels, comprising: assessing a test agent that modulates the cleavage of APP that produces one or more A β peptides, the processing of APP, the processing of A β and/or the level of one or more A β peptides to determine if it affects the cleavage and/or processing of APP and/or portion(s) thereof other than the processing of APP or portion(s) thereof that produces one or more A β peptides; and identifying an agent that modulates A β levels without substantially altering the cleavage and/or processing of APP and/or portion(s) thereof other than the processing of APP or portion(s) thereof that produces one or more A β peptides.

- 1 -

SEQUENCE LISTING

<110> Kounnas, Maria
 Patrick, Aaron
 Wagner, Steven
 Velicelebi, Gonul

<120> METHODS AND COMPOSITIONS FOR MODULATING AMYLOID BETA

<130> 37481-3316PC

<140> Not Yet Assigned

<141> Herewith

<160> 100

<170> FastSEQ for Windows Version 4.0

<210> 1

<211> 3579

<212> DNA

<213> Homo sapiens

<220>

<221> CDS

<222> (148)...(2460)

<223> APP nucleotide sequence encoding Amyloid Beta (A4)
 Precursor Protein

<400> 1

agtttcctcg gcagcggttag gcgagagcac gcggaggagc gtgcgcgggg gccccgggag

60

acggcggcgg tggcggcgcg ggcagagcaa ggacgcggcg gatccactc gcacagcagc

120

gcactcgggtg ccccgcgagc ggtcgcg atg ctg ccc ggt ttg gca ctg ctg ctg

174

Met Leu Pro Gly Leu Ala Leu Leu Leu

1

5

ctg gcc gcc tgg acg gct cgg gcg ctg gag gta ccc act gat ggt aat

222

Leu Ala Ala Trp Thr Ala Arg Ala Leu Glu Val Pro Thr Asp Gly Asn

10

15

20

25

gct ggc ctg ctg gct gaa ccc cag att gcc atg ttc tgt ggc aga ctg

270

Ala Gly Leu Leu Ala Glu Pro Gln Ile Ala Met Phe Cys Gly Arg Leu

30

35

40

- 2 -

aac atg cac atg aat gtc cag aat ggg aag tgg gat tca gat cca tca
 318
 Asn Met His Met Asn Val Gln Asn Gly Lys Trp Asp Ser Asp Pro Ser
 45 50 55

ggg acc aaa acc tgc att gat acc aag gaa ggc atc ctg cag tat tgc
 366
 Gly Thr Lys Thr Cys Ile Asp Thr Lys Glu Gly Ile Leu Gln Tyr Cys
 60 65 70

caa gaa gtc tac cct gaa ctg cag atc acc aat gtg gta gaa gcc aac
 414
 Gln Glu Val Tyr Pro Glu Leu Gln Ile Thr Asn Val Val Glu Ala Asn
 75 80 85

caa cca gtg acc atc cag aac tgg tgc aag cgg ggc cgc aag cag tgc
 462
 Gln Pro Val Thr Ile Gln Asn Trp Cys Lys Arg Gly Arg Lys Gln Cys
 90 95 100 105

aag acc cat ccc cac ttt gtg att ccc tac cgc tgc tta gtt ggt gag
 510
 Lys Thr His Pro His Phe Val Ile Pro Tyr Arg Cys Leu Val Gly Glu
 110 115 120

ttt gta agt gat gcc ctt ctc gtt cct gac aag tgc aaa ttc tta cac
 558
 Phe Val Ser Asp Ala Leu Leu Val Pro Asp Lys Cys Lys Phe Leu His
 125 130 135

cag gag agg atg gat gtt tgc gaa act cat ctt cac tgg cac acc gtc
 606
 Gln Glu Arg Met Asp Val Cys Glu Thr His Leu His Trp His Thr Val
 140 145 150

gcc aaa gag aca tgc agt gag aag agt acc aac ttg cat gac tac ggc
 654
 Ala Lys Glu Thr Cys Ser Glu Lys Ser Thr Asn Leu His Asp Tyr Gly
 155 160 165

atg ttg ctg ccc tgc gga att gac aag ttc cga ggg gta gag ttt gtg
 702
 Met Leu Leu Pro Cys Gly Ile Asp Lys Phe Arg Gly Val Glu Phe Val
 170 175 180 185

tgt tgc cca ctg gct gaa gaa agt gac aat gtg gat tct gct gat gcg
 750
 Cys Cys Pro Leu Ala Glu Glu Ser Asp Asn Val Asp Ser Ala Asp Ala
 190 195 200

gag gag gat gac tcg gat gtc tgg tgg ggc gga gca gac aca gac tat
 798

- 3 -

Glu Glu Asp Asp Ser Asp Val Trp Trp Gly Gly Ala Asp Thr Asp Tyr
 205 210 215
 gca gat ggg agt gaa gac aaa gta gta gaa gta gca gag gag gaa gaa
 846
 Ala Asp Gly Ser Glu Asp Lys Val Val Glu Val Ala Glu Glu Glu Glu
 220 225 230
 gtg gct gag gtg gaa gaa gaa gaa gcc gat gat gac gag gac gat gag
 894
 Val Ala Glu Val Glu Glu Glu Glu Ala Asp Asp Asp Glu Asp Asp Glu
 235 240 245
 gat ggt gat gag gta gag gaa gag gct gag gaa ccc tac gaa gaa gcc
 942
 Asp Gly Asp Glu Val Glu Glu Glu Ala Glu Glu Pro Tyr Glu Glu Ala
 250 255 260 265
 aca gag aga acc acc agc att gcc acc acc acc acc acc acc aca gag
 990
 Thr Glu Arg Thr Thr Ser Ile Ala Thr Thr Thr Thr Thr Thr Thr Glu
 270 275 280
 tct gtg gaa gag gtg gtt cga gag gtg tgc tct gaa caa gcc gag acg
 1038
 Ser Val Glu Glu Val Val Arg Glu Val Cys Ser Glu Gln Ala Glu Thr
 285 290 295
 ggg ccg tgc cga gca atg atc tcc cgc tgg tac ttt gat gtg act gaa
 1086
 Gly Pro Cys Arg Ala Met Ile Ser Arg Trp Tyr Phe Asp Val Thr Glu
 300 305 310
 ggg aag tgt gcc cca ttc ttt tac ggc gga tgt ggc ggc aac cgg aac
 1134
 Gly Lys Cys Ala Pro Phe Phe Tyr Gly Gly Cys Gly Gly Asn Arg Asn
 315 320 325
 aac ttt gac aca gaa gag tac tgc atg gcc gtg tgt ggc agc gcc atg
 1182
 Asn Phe Asp Thr Glu Glu Tyr Cys Met Ala Val Cys Gly Ser Ala Met
 330 335 340 345
 tcc caa agt tta ctc aag act acc cag gaa cct ctt gcc cga gat cct
 1230
 Ser Gln Ser Leu Leu Lys Thr Thr Gln Glu Pro Leu Ala Arg Asp Pro
 350 355 360
 gtt aaa ctt cct aca aca gca gcc agt acc cct gat gcc gtt gac aag
 1278
 Val Lys Leu Pro Thr Thr Ala Ala Ser Thr Pro Asp Ala Val Asp Lys
 365 370 375

- 4 -

tat ctc gag aca cct ggg gat gag aat gaa cat gcc cat ttc cag aaa
 1326
 Tyr Leu Glu Thr Pro Gly Asp Glu Asn Glu His Ala His Phe Gln Lys
 380 385 390

gcc aaa gag agg ctt gag gcc aag cac cga gag aga atg tcc cag gtc
 1374
 Ala Lys Glu Arg Leu Glu Ala Lys His Arg Glu Arg Met Ser Gln Val
 395 400 405

atg aga gaa tgg gaa gag gca gaa cgt caa gca aag aac ttg cct aaa
 1422
 Met Arg Glu Trp Glu Glu Ala Glu Arg Gln Ala Lys Asn Leu Pro Lys
 410 415 420 425

gct gat aag aag gca gtt atc cag cat ttc cag gag aaa gtg gaa tct
 1470
 Ala Asp Lys Lys Ala Val Ile Gln His Phe Gln Glu Lys Val Glu Ser
 430 435 440

ttg gaa cag gaa gca gcc aac gag aga cag cag ctg gtg gag aca cac
 1518
 Leu Glu Gln Glu Ala Ala Asn Glu Arg Gln Gln Leu Val Glu Thr His
 445 450 455

atg gcc aga gtg gaa gcc atg ctc aat gac cgc cgc cgc ctg gcc ctg
 1566
 Met Ala Arg Val Glu Ala Met Leu Asn Asp Arg Arg Arg Leu Ala Leu
 460 465 470

gag aac tac atc acc gct ctg cag gct gtt cct cct cgg cct cgt cac
 1614
 Glu Asn Tyr Ile Thr Ala Leu Gln Ala Val Pro Pro Arg Pro Arg His
 475 480 485

gtg ttc aat atg cta aag aag tat gtc cgc gca gaa cag aag gac aga
 1662
 Val Phe Asn Met Leu Lys Lys Tyr Val Arg Ala Glu Gln Lys Asp Arg
 490 495 500 505

cag cac acc cta aag cat ttc gag cat gtg cgc atg gtg gat ccc aag
 1710
 Gln His Thr Leu Lys His Phe Glu His Val Arg Met Val Asp Pro Lys
 510 515 520

aaa gcc gct cag atc cgg tcc cag gtt atg aca cac ctc cgt gtg att
 1758
 Lys Ala Ala Gln Ile Arg Ser Gln Val Met Thr His Leu Arg Val Ile
 525 530 535

- 5 -

tat gag cgc atg aat cag tct ctc tcc ctg ctc tac aac gtg cct gca
1806

Tyr Glu Arg Met Asn Gln Ser Leu Ser Leu Leu Tyr Asn Val Pro Ala
540 545 550

gtg gcc gag gag att cag gat gaa gtt gat gag ctg ctt cag aaa gag
1854

Val Ala Glu Glu Ile Gln Asp Glu Val Asp Glu Leu Leu Gln Lys Glu
555 560 565

caa aac tat tca gat gac gtc ttg gcc aac atg att agt gaa cca agg
1902

Gln Asn Tyr Ser Asp Asp Val Leu Ala Asn Met Ile Ser Glu Pro Arg
570 575 580 585

atc agt tac gga aac gat gct ctc atg cca tct ttg acc gaa acg aaa
1950

Ile Ser Tyr Gly Asn Asp Ala Leu Met Pro Ser Leu Thr Glu Thr Lys
590 595 600

acc acc gtg gag ctc ctt ccc gtg aat gga gag ttc agc ctg gac gat
1998

Thr Thr Val Glu Leu Leu Pro Val Asn Gly Glu Phe Ser Leu Asp Asp
605 610 615

ctc cag ccg tgg cat tct ttt ggg gct gac tct gtg cca gcc aac aca
2046

Leu Gln Pro Trp His Ser Phe Gly Ala Asp Ser Val Pro Ala Asn Thr
620 625 630

gaa aac gaa gtt gag cct gtt gat gcc cgc cct gct gcc gac cga gga
2094

Glu Asn Glu Val Glu Pro Val Asp Ala Arg Pro Ala Ala Asp Arg Gly
635 640 645

ctg acc act cga cca ggt tct ggg ttg aca aat atc aag acg gag gag
2142

Leu Thr Thr Arg Pro Gly Ser Gly Leu Thr Asn Ile Lys Thr Glu Glu
650 655 660 665

atc tct gaa gtg aag atg gat gca gaa ttc cga cat gac tca gga tat
2190

Ile Ser Glu Val Lys Met Asp Ala Glu Phe Arg His Asp Ser Gly Tyr
670 675 680

gaa gtt cat cat caa aaa ttg gtg ttc ttt gca gaa gat gtg ggt tca
2238

Glu Val His His Gln Lys Leu Val Phe Phe Ala Glu Asp Val Gly Ser
685 690 695

aac aaa ggt gca atc att gga ctc atg gtg ggc ggt gtt gtc ata gcg
2286

- 6 -

Asn Lys Gly Ala Ile Ile Gly Leu Met Val Gly Gly Val Val Ile Ala
 700 705 710
 aca gtg atc gtc atc acc ttg gtg atg ctg aag aag aaa cag tac aca
 2334
 Thr Val Ile Val Ile Thr Leu Val Met Leu Lys Lys Lys Gln Tyr Thr
 715 720 725
 tcc att cat cat ggt gtg gtg gag gtt gac gcc gct gtc acc cca gag
 2382
 Ser Ile His His Gly Val Val Glu Val Asp Ala Ala Val Thr Pro Glu
 730 735 740 745
 gag cgc cac ctg tcc aag atg cag cag aac ggc tac gaa aat cca acc
 2430
 Glu Arg His Leu Ser Lys Met Gln Gln Asn Gly Tyr Glu Asn Pro Thr
 750 755 760
 tac aag ttc ttt gag cag atg cag aac tag acccccgcca cagcagcctc
 2480
 Tyr Lys Phe Phe Glu Gln Met Gln Asn
 765 770
 tgaagttgga cagcaaaacc attgcttcac taccatcgg tgtccattta tagaataatg
 2540
 tgggaagaaa caaaccggtt ttatgattta ctcatatcg ccttttgaca gctgtgctgt
 2600
 aacacaagta gatgctgaa cttgaattaa tccacacatc agtaatgtat tctatctctc
 2660
 tttacatctt ggtctctata ctacattatt aatgggtttt gtgtactgta aagaatttag
 2720
 ctgtatcaaa ctagtgcatt aatagattct ctctgatta tttatcacat agccccttag
 2780
 ccagttgtat attattcttg tggtttgtga cccaattaag tcctacttta catatgcttt
 2840
 aagaatcgat gggggatgct tcatgtgaac gtgggagttc agctgcttct cttgcctaag
 2900
 tattcctttc ctgatcacta tgcattttta agttaaacat ttttaagtat ttcagatgct
 2960
 ttagagagat tttttttcca tgactgcatt ttactgtaca gattgctgct tctgctatat
 3020
 ttgtgatata ggaattaaga ggatacacac gtttggtttct tcgtgcctgt tttatgtgca
 3080
 cacattagga attgagactt caagcttttc tttttttgtc cacgtatctt tgggtctttg
 3140
 ataaagaaaa gaatccctgt tcattgtaag cacttttacg gggcggtgg ggaggggtgc
 3200
 tctgctgggc ttcaattacc aagaattctc caaaacaatt ttctgcagga tgattgtaca
 3260
 gaatcattgc ttatgacatg atcgctttct acactgtatt acataaataa attaaataaa
 3320

- 7 -

ataaccccg gcaagacttt tctttgaagg atgactacag acattaaata atcgaagtaa
 3380
 ttttgggtgg ggagaagagg cagattcaat tttctttaac cagtctgaag tttcatttat
 3440
 gatacaaaaag aagatgaaaa tggaagtggc aatataaggg gatgaggaag gcatgcctgg
 3500
 acaaaccctt cttttaagat gtgtcttcaa tttgtataaa atgggtgttt catgtaaata
 3560
 aatacattct tggaggagc
 3579

<210> 2
 <211> 770
 <212> PRT
 <213> Homo sapiens

<400> 2
 Met Leu Pro Gly Leu Ala Leu Leu Leu Leu Ala Ala Trp Thr Ala Arg
 1 5 10 15
 Ala Leu Glu Val Pro Thr Asp Gly Asn Ala Gly Leu Leu Ala Glu Pro
 20 25 30
 Gln Ile Ala Met Phe Cys Gly Arg Leu Asn Met His Met Asn Val Gln
 35 40 45
 Asn Gly Lys Trp Asp Ser Asp Pro Ser Gly Thr Lys Thr Cys Ile Asp
 50 55 60
 Thr Lys Glu Gly Ile Leu Gln Tyr Cys Gln Glu Val Tyr Pro Glu Leu
 65 70 75 80
 Gln Ile Thr Asn Val Val Glu Ala Asn Gln Pro Val Thr Ile Gln Asn
 85 90 95
 Trp Cys Lys Arg Gly Arg Lys Gln Cys Lys Thr His Pro His Phe Val
 100 105 110
 Ile Pro Tyr Arg Cys Leu Val Gly Glu Phe Val Ser Asp Ala Leu Leu
 115 120 125
 Val Pro Asp Lys Cys Lys Phe Leu His Gln Glu Arg Met Asp Val Cys
 130 135 140
 Glu Thr His Leu His Trp His Thr Val Ala Lys Glu Thr Cys Ser Glu
 145 150 155 160
 Lys Ser Thr Asn Leu His Asp Tyr Gly Met Leu Leu Pro Cys Gly Ile
 165 170 175
 Asp Lys Phe Arg Gly Val Glu Phe Val Cys Cys Pro Leu Ala Glu Glu
 180 185 190
 Ser Asp Asn Val Asp Ser Ala Asp Ala Glu Glu Asp Asp Ser Asp Val
 195 200 205
 Trp Trp Gly Gly Ala Asp Thr Asp Tyr Ala Asp Gly Ser Glu Asp Lys
 210 215 220
 Val Val Glu Val Ala Glu Glu Glu Val Ala Glu Val Glu Glu Glu
 225 230 235 240
 Glu Ala Asp Asp Asp Glu Asp Asp Glu Asp Gly Asp Glu Val Glu Glu
 245 250 255
 Glu Ala Glu Glu Pro Tyr Glu Glu Ala Thr Glu Arg Thr Thr Ser Ile
 260 265 270
 Ala Thr Thr Thr Thr Thr Thr Thr Glu Ser Val Glu Glu Val Val Arg

		275						280						285				
Glu	Val	Cys	Ser	Glu	Gln	Ala	Glu	Thr	Gly	Pro	Cys	Arg	Ala	Met	Ile			
	290					295					300							
Ser	Arg	Trp	Tyr	Phe	Asp	Val	Thr	Glu	Gly	Lys	Cys	Ala	Pro	Phe	Phe			
305					310					315					320			
Tyr	Gly	Gly	Cys	Gly	Gly	Asn	Arg	Asn	Asn	Phe	Asp	Thr	Glu	Glu	Tyr			
				325					330					335				
Cys	Met	Ala	Val	Cys	Gly	Ser	Ala	Met	Ser	Gln	Ser	Leu	Leu	Lys	Thr			
			340					345				350						
Thr	Gln	Glu	Pro	Leu	Ala	Arg	Asp	Pro	Val	Lys	Leu	Pro	Thr	Thr	Ala			
		355				360					365							
Ala	Ser	Thr	Pro	Asp	Ala	Val	Asp	Lys	Tyr	Leu	Glu	Thr	Pro	Gly	Asp			
	370					375					380							
Glu	Asn	Glu	His	Ala	His	Phe	Gln	Lys	Ala	Lys	Glu	Arg	Leu	Glu	Ala			
385					390					395					400			
Lys	His	Arg	Glu	Arg	Met	Ser	Gln	Val	Met	Arg	Glu	Trp	Glu	Glu	Ala			
				405				410					415					
Glu	Arg	Gln	Ala	Lys	Asn	Leu	Pro	Lys	Ala	Asp	Lys	Lys	Ala	Val	Ile			
		420						425				430						
Gln	His	Phe	Gln	Glu	Lys	Val	Glu	Ser	Leu	Glu	Gln	Glu	Ala	Ala	Asn			
	435					440					445							
Glu	Arg	Gln	Gln	Leu	Val	Glu	Thr	His	Met	Ala	Arg	Val	Glu	Ala	Met			
	450					455				460								
Leu	Asn	Asp	Arg	Arg	Arg	Leu	Ala	Leu	Glu	Asn	Tyr	Ile	Thr	Ala	Leu			
465					470					475					480			
Gln	Ala	Val	Pro	Pro	Arg	Pro	Arg	His	Val	Phe	Asn	Met	Leu	Lys	Lys			
				485					490				495					
Tyr	Val	Arg	Ala	Glu	Gln	Lys	Asp	Arg	Gln	His	Thr	Leu	Lys	His	Phe			
		500						505				510						
Glu	His	Val	Arg	Met	Val	Asp	Pro	Lys	Lys	Ala	Ala	Gln	Ile	Arg	Ser			
	515					520					525							
Gln	Val	Met	Thr	His	Leu	Arg	Val	Ile	Tyr	Glu	Arg	Met	Asn	Gln	Ser			
	530					535					540							
Leu	Ser	Leu	Leu	Tyr	Asn	Val	Pro	Ala	Val	Ala	Glu	Glu	Ile	Gln	Asp			
545					550					555					560			
Glu	Val	Asp	Glu	Leu	Leu	Gln	Lys	Glu	Gln	Asn	Tyr	Ser	Asp	Asp	Val			
				565					570				575					
Leu	Ala	Asn	Met	Ile	Ser	Glu	Pro	Arg	Ile	Ser	Tyr	Gly	Asn	Asp	Ala			
		580						585				590						
Leu	Met	Pro	Ser	Leu	Thr	Glu	Thr	Lys	Thr	Thr	Val	Glu	Leu	Leu	Pro			
	595					600					605							
Val	Asn	Gly	Glu	Phe	Ser	Leu	Asp	Asp	Leu	Gln	Pro	Trp	His	Ser	Phe			
	610					615				620								
Gly	Ala	Asp	Ser	Val	Pro	Ala	Asn	Thr	Glu	Asn	Glu	Val	Glu	Pro	Val			
625					630													

- 9 -

```

        690              695              700
Leu Met Val Gly Gly Val Val Ile Ala Thr Val Ile Val Ile Thr Leu
705              710              715              720
Val Met Leu Lys Lys Lys Gln Tyr Thr Ser Ile His His Gly Val Val
              725              730              735
Glu Val Asp Ala Ala Val Thr Pro Glu Glu Arg His Leu Ser Lys Met
              740              745              750
Gln Gln Asn Gly Tyr Glu Asn Pro Thr Tyr Lys Phe Phe Glu Gln Met
              755              760              765
Gln Asn
              770

```

<210> 3
 <211> 248
 <212> DNA
 <213> Homo sapiens

<220>
 <221> CDS
 <222> (51)...(179)
 <223> nucleotide sequence encoding Human amyloid-beta
 precursor protein

<400> 3
 gttctggggtt gacaaatattc aagacggagg agatctctga agtgaagatg gat gca
 56

Asp Ala
 1

```

gaa ttc cga cat gac tca gga tat gaa gtt cat cat caa aaa ttg gtg
104
Glu Phe Arg His Asp Ser Gly Tyr Glu Val His His Gln Lys Leu Val
      5              10              15

```

```

ttc ttt gca gaa gat gtg ggt tca aac aaa ggt gca atc att gga ctc
152
Phe Phe Ala Glu Asp Val Gly Ser Asn Lys Gly Ala Ile Ile Gly Leu
      20              25              30

```

```

atg gtg ggc ggt gtt gtc ata gcg aca gtgacgtca tcaccttggt
199
Met Val Gly Gly Val Val Ile Ala Thr
      35              40

```

```

gatgctgaag aagaaacagt acacatccat tcatcatggt gtggtggag
248

```

<210> 4
 <211> 43
 <212> PRT
 <213> Homo sapiens

- 10 -

<400> 4

Asp	Ala	Glu	Phe	Arg	His	Asp	Ser	Gly	Tyr	Glu	Val	His	His	Gln	Lys
1				5				10						15	
Leu	Val	Phe	Phe	Ala	Glu	Asp	Val	Gly	Ser	Asn	Lys	Gly	Ala	Ile	Ile
			20					25					30		
Gly	Leu	Met	Val	Gly	Gly	Val	Val	Ile	Ala	Thr					
		35					40								

<210> 5

<211> 2765

<212> DNA

<213> Homo sapiens

<220>

<221> CDS

<222> (249)...(1649)

<223> nucleotide sequence encoding human Presenilin-1
(hPS1)

<400> 5

tgggacagggc agctccgggg tccgcgggttt cacatcggaa acaaaacagc ggctgggtctg
 60
 gaaggaacct gagctacgag ccgcgggcggc agcggggcgg cggggaagcg tataccta
 120
 ctgggagcct gcaagtgaca acagcctttg cggtccttag acagcttggc ctggaggaga
 180
 acacatgaaa gaaagaacct caagaggctt tgttttctgt gaaacagtat ttctatacag
 240
 ttgtccca atg aca gag tta cct gca ccg ttg tcc tac ttc cag aat gca
 290

Met	Thr	Glu	Leu	Pro	Ala	Pro	Leu	Ser	Tyr	Phe	Gln	Asn	Ala
1				5						10			

cag atg tct gag gac aac cac ctg agc aat act gta cgt agc cag aat
 338

Gln	Met	Ser	Glu	Asp	Asn	His	Leu	Ser	Asn	Thr	Val	Arg	Ser	Gln	Asn
15					20					25				30	

gac aat aga gaa cgg cag gag cac aac gac aga cgg agc ctt ggc cac
 386

Asp	Asn	Arg	Glu	Arg	Gln	Glu	His	Asn	Asp	Arg	Arg	Ser	Leu	Gly	His
				35					40					45	

cct gag cca tta tct aat gga cga ccc cag ggt aac tcc cgg cag gtg
 434

Pro	Glu	Pro	Leu	Ser	Asn	Gly	Arg	Pro	Gln	Gly	Asn	Ser	Arg	Gln	Val
			50					55					60		

gtg gag caa gat gag gaa gaa gat gag gag ctg aca ttg aaa tat ggc
 482

Val	Glu	Gln	Asp	Glu	Glu	Glu	Asp	Glu	Glu	Leu	Thr	Leu	Lys	Tyr	Gly
			65				70						75		

- 11 -

gcc aag cat gtg atc atg ctc ttt gtc cct gtg act ctc tgc atg gtg
530
Ala Lys His Val Ile Met Leu Phe Val Pro Val Thr Leu Cys Met Val
80 85 90

gtg gtc gtg gct acc att aag tca gtc agc ttt tat acc cgg aag gat
578
Val Val Val Ala Thr Ile Lys Ser Val Ser Phe Tyr Thr Arg Lys Asp
95 100 105 110

ggg cag cta atc tat acc cca ttc aca gaa gat acc gag act gtg ggc
626
Gly Gln Leu Ile Tyr Thr Pro Phe Thr Glu Asp Thr Glu Thr Val Gly
115 120 125

cag aga gcc ctg cac tca att ctg aat gct gcc atc atg atc agt gtc
674
Gln Arg Ala Leu His Ser Ile Leu Asn Ala Ala Ile Met Ile Ser Val
130 135 140

att gtt gtc atg act atc ctc ctg gtg gtt ctg tat aaa tac agg tgc
722
Ile Val Val Met Thr Ile Leu Leu Val Val Leu Tyr Lys Tyr Arg Cys
145 150 155

tat aag gtc atc cat gcc tgg ctt att ata tca tct cta ttg ttg ctg
770
Tyr Lys Val Ile His Ala Trp Leu Ile Ile Ser Ser Leu Leu Leu Leu
160 165 170

ttc ttt ttt tca ttc att tac ttg ggg gaa gtg ttt aaa acc tat aac
818
Phe Phe Phe Ser Phe Ile Tyr Leu Gly Glu Val Phe Lys Thr Tyr Asn
175 180 185 190

gtt gct gtg gac tac att act gtt gca ctc ctg atc tgg aat ttt ggt
866
Val Ala Val Asp Tyr Ile Thr Val Ala Leu Leu Ile Trp Asn Phe Gly
195 200 205

gtg gtg gga atg att tcc att cac tgg aaa ggt cca ctt cga ctc cag
914
Val Val Gly Met Ile Ser Ile His Trp Lys Gly Pro Leu Arg Leu Gln
210 215 220

cag gca tat ctc att atg att agt gcc ctc atg gcc ctg gtg ttt atc
962
Gln Ala Tyr Leu Ile Met Ile Ser Ala Leu Met Ala Leu Val Phe Ile
225 230 235

- 12 -

aag tac ctc cct gaa tgg act gcg tgg ctc atc ttg gct gtg att tca
1010

Lys Tyr Leu Pro Glu Trp Thr Ala Trp Leu Ile Leu Ala Val Ile Ser
240 245 250

gta tat gat tta gtg gct gtt ttg tgt ccg aaa ggt cca ctt cgt atg
1058

Val Tyr Asp Leu Val Ala Val Leu Cys Pro Lys Gly Pro Leu Arg Met
255 260 265 270

ctg gtt gaa aca gct cag gag aga aat gaa acg ctt ttt cca gct ctc
1106

Leu Val Glu Thr Ala Gln Glu Arg Asn Glu Thr Leu Phe Pro Ala Leu
275 280 285

att tac tcc tca aca atg gtg tgg ttg gtg aat atg gca gaa gga gac
1154

Ile Tyr Ser Ser Thr Met Val Trp Leu Val Asn Met Ala Glu Gly Asp
290 295 300

ccg gaa gct caa agg aga gta tcc aaa aat tcc aag tat aat gca gaa
1202

Pro Glu Ala Gln Arg Arg Val Ser Lys Asn Ser Lys Tyr Asn Ala Glu
305 310 315

agc aca gaa agg gag tca caa gac act gtt gca gag aat gat gat ggc
1250

Ser Thr Glu Arg Glu Ser Gln Asp Thr Val Ala Glu Asn Asp Asp Gly
320 325 330

ggg ttc agt gag gaa tgg gaa gcc cag agg gac agt cat cta ggg cct
1298

Gly Phe Ser Glu Glu Trp Glu Ala Gln Arg Asp Ser His Leu Gly Pro
335 340 345 350

cat cgc tct aca cct gag tca cga gct gct gtc cag gaa ctt tcc agc
1346

His Arg Ser Thr Pro Glu Ser Arg Ala Ala Val Gln Glu Leu Ser Ser
355 360 365

agt atc ctc gct ggt gaa gac cca gag gaa agg gga gta aaa ctt gga
1394

Ser Ile Leu Ala Gly Glu Asp Pro Glu Glu Arg Gly Val Lys Leu Gly
370 375 380

ttg gga gat ttc att ttc tac agt gtt ctg gtt ggt aaa gcc tca gca
1442

Leu Gly Asp Phe Ile Phe Tyr Ser Val Leu Val Gly Lys Ala Ser Ala
385 390 395

aca gcc agt gga gac tgg aac aca acc ata gcc tgt ttc gta gcc ata
1490

- 13 -

Thr Ala Ser Gly Asp Trp Asn Thr Thr Ile Ala Cys Phe Val Ala Ile
 400 405 410
 tta att ggt ttg tgc ctt aca tta tta ctc ctt gcc att ttc aag aaa
 1538
 Leu Ile Gly Leu Cys Leu Thr Leu Leu Leu Leu Ala Ile Phe Lys Lys
 415 420 425 430
 gca ttg cca gct ctt cca atc tcc atc acc ttt ggg ctt gtt ttc tac
 1586
 Ala Leu Pro Ala Leu Pro Ile Ser Ile Thr Phe Gly Leu Val Phe Tyr
 435 440 445
 ttt gcc aca gat tat ctt gta cag cct ttt atg gac caa tta gca ttc
 1634
 Phe Ala Thr Asp Tyr Leu Val Gln Pro Phe Met Asp Gln Leu Ala Phe
 450 455 460
 cat caa ttt tat atc tagcatatctt ggggttagaa tcccatggat gtttcttctt
 1689
 His Gln Phe Tyr Ile
 465
 tgactataac caaatctggg gaggacaaag gtgattttcc tgtgtccaca tctaacaag
 1749
 tcaagattcc cggttgact tttgcagctt ccttccaagt cttcctgacc accttgact
 1809
 attggacttt ggaaggaggt gcctatagaa aacgattttg aacatacttc atcgagtg
 1869
 actgtgtccc tcggtgcaga aactaccaga tttgaggagc gaggtcaagg agatatgata
 1929
 ggcccgaag ttgctgtgcc ccatcagcag cttgacgcgt ggtcacagga cgatttcact
 1989
 gacactgca actctcagga ctaccggtta ccaagaggtt aggtgaagtg gtttaaacca
 2049
 aacggaactc ttcattctaa actacacgtt gaaaatcaac ccaataattc tgtattaact
 2109
 gaattctgaa cttttcagga ggtactgtga ggaagagcag gcaccagcag cagaatggg
 2169
 aatggagagg tgggcagggg ttccagcttc cctttgattt tttgctgcag actcatcctt
 2229
 tttaaatgag acttggtttc cctctctttt gagtcaagtc aaatatgtag attgcctttg
 2289
 gcaattcttc ttctcaagca ctgacactca ttaccgtctg tgattgccat ttcttccaa
 2349
 ggccagtctg aacctgaggt tgctttatcc taaaagtttt aacctcaggt tccaaattca
 2409
 gtaaattttg gaaacagtac agctatttct catcaattct ctatcatgtt gaagtcaaat
 2469
 ttggattttc caccaaattc tgaatttgta gacatacttg tacgctcact tgccccaga
 2529

- 14 -

tgcctcctct gtcctcattc ttctctccca cacaagcagt ctttttctac agccagtaag
 2589
 gcagctctgt crtggtagca gatgggccca ttattctagg gtcttactct ttgtatgatg
 2649
 aaaagaatgt gttatgaatc ggtgctgtca gccctgctgt cagaccttct tccacagcaa
 2709
 atgagatgta tgcccaaagc ggtagaatta aagaagagta aaatggctgt tgaagc
 2765

<210> 6

<211> 467

<212> PRT

<213> Homo sapiens

<400> 6

Met	Thr	Glu	Leu	Pro	Ala	Pro	Leu	Ser	Tyr	Phe	Gln	Asn	Ala	Gln	Met
1				5					10					15	
Ser	Glu	Asp	Asn	His	Leu	Ser	Asn	Thr	Val	Arg	Ser	Gln	Asn	Asp	Asn
			20					25					30		
Arg	Glu	Arg	Gln	Glu	His	Asn	Asp	Arg	Arg	Ser	Leu	Gly	His	Pro	Glu
			35				40					45			
Pro	Leu	Ser	Asn	Gly	Arg	Pro	Gln	Gly	Asn	Ser	Arg	Gln	Val	Val	Glu
			50			55				60					
Gln	Asp	Glu	Glu	Glu	Asp	Glu	Glu	Leu	Thr	Leu	Lys	Tyr	Gly	Ala	Lys
65					70					75					80
His	Val	Ile	Met	Leu	Phe	Val	Pro	Val	Thr	Leu	Cys	Met	Val	Val	Val
				85					90					95	
Val	Ala	Thr	Ile	Lys	Ser	Val	Ser	Phe	Tyr	Thr	Arg	Lys	Asp	Gly	Gln
			100					105					110		
Leu	Ile	Tyr	Thr	Pro	Phe	Thr	Glu	Asp	Thr	Glu	Thr	Val	Gly	Gln	Arg
			115				120					125			
Ala	Leu	His	Ser	Ile	Leu	Asn	Ala	Ala	Ile	Met	Ile	Ser	Val	Ile	Val
						135						140			
Val	Met	Thr	Ile	Leu	Leu	Val	Val	Leu	Tyr	Lys	Tyr	Arg	Cys	Tyr	Lys
145					150					155					160
Val	Ile	His	Ala	Trp	Leu	Ile	Ile	Ser	Ser	Leu	Leu	Leu	Leu	Phe	Phe
				165					170					175	
Phe	Ser	Phe	Ile	Tyr	Leu	Gly	Glu	Val	Phe	Lys	Thr	Tyr	Asn	Val	Ala
			180				185						190		
Val	Asp	Tyr	Ile	Thr	Val	Ala	Leu	Leu	Ile	Trp	Asn	Phe	Gly	Val	Val
			195				200					205			
Gly	Met	Ile	Ser	Ile	His	Trp	Lys	Gly	Pro	Leu	Arg	Leu	Gln	Gln	Ala
						215					220				
Tyr	Leu	Ile	Met	Ile	Ser	Ala	Leu	Met	Ala	Leu	Val	Phe	Ile	Lys	Tyr
225					230					235					240
Leu	Pro	Glu	Trp	Thr	Ala	Trp	Leu	Ile	Leu	Ala	Val	Ile	Ser	Val	Tyr
				245					250					255	
Asp	Leu	Val	Ala	Val	Leu	Cys	Pro	Lys	Gly	Pro	Leu	Arg	Met	Leu	Val
			260					265					270		
Glu	Thr	Ala	Gln	Glu	Arg	Asn	Glu	Thr	Leu	Phe	Pro	Ala	Leu	Ile	Tyr
			275				280					285			
Ser	Ser	Thr	Met	Val	Trp	Leu	Val	Asn	Met	Ala	Glu	Gly	Asp	Pro	Glu

- 15 -

```

      290              295              300
Ala Gln Arg Arg Val Ser Lys Asn Ser Lys Tyr Asn Ala Glu Ser Thr
305              310              315              320
Glu Arg Glu Ser Gln Asp Thr Val Ala Glu Asn Asp Asp Gly Gly Phe
      325              330              335
Ser Glu Glu Trp Glu Ala Gln Arg Asp Ser His Leu Gly Pro His Arg
      340              345              350
Ser Thr Pro Glu Ser Arg Ala Ala Val Gln Glu Leu Ser Ser Ser Ile
      355              360              365
Leu Ala Gly Glu Asp Pro Glu Glu Arg Gly Val Lys Leu Gly Leu Gly
      370              375              380
Asp Phe Ile Phe Tyr Ser Val Leu Val Gly Lys Ala Ser Ala Thr Ala
385              390              395              400
Ser Gly Asp Trp Asn Thr Thr Ile Ala Cys Phe Val Ala Ile Leu Ile
      405              410              415
Gly Leu Cys Leu Thr Leu Leu Leu Leu Ala Ile Phe Lys Lys Ala Leu
      420              425              430
Pro Ala Leu Pro Ile Ser Ile Thr Phe Gly Leu Val Phe Tyr Phe Ala
      435              440              445
Thr Asp Tyr Leu Val Gln Pro Phe Met Asp Gln Leu Ala Phe His Gln
      450              455              460
Phe Tyr Ile
465

```

<210> 7

<211> 2236

<212> DNA

<213> Homo sapiens

<220>

<221> CDS

<222> (368)...(1714)

<223> nucleotide sequence encoding human presenilin-2
(PSEN2)

<400> 7

```

cgagcggcgg cggagcaggc atttccagca gtgaggagac agccagaagc aagctattgg
60
agctgaagga acctgagaca gaagctagtc cccctctga attttactga tgaagaaact
120
gaggccacag agctaaagtg acttttccca aggtcgccca gcgaggacgt gggacttctc
180
agacgtcagg agagtgatgt gagggagctg tgtgaccata gaaagtgacg tgttaaaaac
240
cagcgctgcc ctctttgaaa gccagggagc atcattcatt tagcctgctg agaagaagaa
300
accaagtgtc cgggattcag acctctctgc ggccccaagt gttcgtggtg cttccagagg
360
cagggct atg ctc aca ttc atg gcc tct gac agc gag gaa gaa gtg tgt
409

```

Met Leu Thr Phe Met Ala Ser Asp Ser Glu Glu Glu Val Cys

1

5

10

- 16 -

gat gag cgg acg tcc cta atg tcg gcc gag agc ccc acg ccg cgc tcc
 457
 Asp Glu Arg Thr Ser Leu Met Ser Ala Glu Ser Pro Thr Pro Arg Ser
 15 20 25 30

tgc cag gag ggc agg cag ggc cca gag gat gga gag aac act gcc cag
 505
 Cys Gln Glu Gly Arg Gln Gly Pro Glu Asp Gly Glu Asn Thr Ala Gln
 35 40 45

tgg aga agc cag gag aac gag gag gac ggt gag gag gac cct gac cgc
 553
 Trp Arg Ser Gln Glu Asn Glu Glu Asp Gly Glu Glu Asp Pro Asp Arg
 50 55 60

tat gtc tgt agt ggg gtt ccc ggg cgg ccg cca ggc ctg gag gaa gag
 601
 Tyr Val Cys Ser Gly Val Pro Gly Arg Pro Pro Gly Leu Glu Glu Glu
 65 70 75

ctg acc ctc aaa tac gga gcg aag cac gtg atc atg ctg ttt gtg cct
 649
 Leu Thr Leu Lys Tyr Gly Ala Lys His Val Ile Met Leu Phe Val Pro
 80 85 90

gtc act ctg tgc atg atc gtg gtg gta gcc acc atc aag tct gtg cgc
 697
 Val Thr Leu Cys Met Ile Val Val Val Ala Thr Ile Lys Ser Val Arg
 95 100 105 110

ttc tac aca gag aag aat gga cag ctc atc tac acg aca ttc act gag
 745
 Phe Tyr Thr Glu Lys Asn Gly Gln Leu Ile Tyr Thr Thr Phe Thr Glu
 115 120 125

gac aca ccc tcg gtg ggc cag cgc ctc ctc aac tcc gtg ctg aac acc
 793
 Asp Thr Pro Ser Val Gly Gln Arg Leu Leu Asn Ser Val Leu Asn Thr
 130 135 140

ctc atc atg atc agc gtc atc gtg gtt atg acc atc ttc ttg gtg gtg
 841
 Leu Ile Met Ile Ser Val Ile Val Val Met Thr Ile Phe Leu Val Val
 145 150 155

ctc tac aag tac cgc tgc tac aag ttc atc cat ggc tgg ttg atc atg
 889
 Leu Tyr Lys Tyr Arg Cys Tyr Lys Phe Ile His Gly Trp Leu Ile Met
 160 165 170

- 17 -

tct tca ctg atg ctg ctg ttc ctc ttc acc tat atc tac ctt ggg gaa
937

Ser Ser Leu Met Leu Leu Phe Leu Phe Thr Tyr Ile Tyr Leu Gly Glu
175 180 185 190

gtg ctc aag acc tac aat gtg gcc atg gac tac ccc acc ctc ttg ctg
985

Val Leu Lys Thr Tyr Asn Val Ala Met Asp Tyr Pro Thr Leu Leu Leu
195 200 205

act gtc tgg aac ttc ggg gca gtg ggc atg gtg tgc atc cac tgg aag
1033

Thr Val Trp Asn Phe Gly Ala Val Gly Met Val Cys Ile His Trp Lys
210 215 220

ggc cct ctg gtg ctg cag cag gcc tac ctc atc atg atc agt gcg ctc
1081

Gly Pro Leu Val Leu Gln Gln Ala Tyr Leu Ile Met Ile Ser Ala Leu
225 230 235

atg gcc cta gtg ttc atc aag tac ctc cca gag tgg tcc gcg tgg gtc
1129

Met Ala Leu Val Phe Ile Lys Tyr Leu Pro Glu Trp Ser Ala Trp Val
240 245 250

atc ctg ggc gcc atc tct gtg tat gat ctc gtg gct gtg ctg tgt ccc
1177

Ile Leu Gly Ala Ile Ser Val Tyr Asp Leu Val Ala Val Leu Cys Pro
255 260 265 270

aaa ggg cct ctg aga atg ctg gta gaa act gcc cag gag aga aat gag
1225

Lys Gly Pro Leu Arg Met Leu Val Glu Thr Ala Gln Glu Arg Asn Glu
275 280 285

ccc ata ttc cct gcc ctg ata tac tca tct gcc atg gtg tgg acg gtt
1273

Pro Ile Phe Pro Ala Leu Ile Tyr Ser Ser Ala Met Val Trp Thr Val
290 295 300

ggc atg gcg aag ctg gac ccc tcc tct cag ggt gcc ctc cag ctc ccc
1321

Gly Met Ala Lys Leu Asp Pro Ser Ser Gln Gly Ala Leu Gln Leu Pro
305 310 315

tac gac ccg gag atg gaa gaa gac tcc tat gac agt ttt ggg gag cct
1369

Tyr Asp Pro Glu Met Glu Glu Asp Ser Tyr Asp Ser Phe Gly Glu Pro
320 325 330

tca tac ccc gaa gtc ttt gag cct ccc ttg act ggc tac cca ggg gag
1417

- 18 -

Ser Tyr Pro Glu Val Phe Glu Pro Pro Leu Thr Gly Tyr Pro Gly Glu
 335 340 345 350

gag ctg gag gaa gag gag gaa agg ggc gtg aag ctt ggc ctc ggg gac
 1465

Glu Leu Glu Glu Glu Glu Arg Gly Val Lys Leu Gly Leu Gly Asp
 355 360 365

ttc atc ttc tac agt gtg ctg gtg ggc aag gcg gct gcc acg ggc agc
 1513

Phe Ile Phe Tyr Ser Val Leu Val Gly Lys Ala Ala Ala Thr Gly Ser
 370 375 380

ggg gac tgg aat acc acg ctg gcc tgc ttc gtg gcc atc ctc att ggc
 1561

Gly Asp Trp Asn Thr Thr Leu Ala Cys Phe Val Ala Ile Leu Ile Gly
 385 390 395

ttg tgt ctg acc ctc ctg ctg ctt gct gtg ttc aag aag gcg ctg ccc
 1609

Leu Cys Leu Thr Leu Leu Leu Leu Ala Val Phe Lys Lys Ala Leu Pro
 400 405 410

gcc ctc ccc atc tcc atc acg ttc ggc ctc atc ttt tac ttc tcc acg
 1657

Ala Leu Pro Ile Ser Ile Thr Phe Gly Leu Ile Phe Tyr Phe Ser Thr
 415 420 425 430

gac aac ctg gtg cgg ccg ttc atg gac acc ctg gcc tcc cat cag ctc
 1705

Asp Asn Leu Val Arg Pro Phe Met Asp Thr Leu Ala Ser His Gln Leu
 435 440 445

tac atc tga gggacatggt gtgccacagg ctgcaagctg cagggaaattt
 1754

Tyr Ile

tcattggatg cagttgtata gttttacact ctagtgccat atatttttaa gacttttctt
 1814

tccttaaaaa ataaagtacg tgtttacttg gtgaggagga ggcagaacca gctctttggt
 1874

gccagctggt tcatcaccag actttggctc ccgctttggg gagcgctcg cttcacggac
 1934

aggaagcaca gcaggtttat ccagatgaac tgagaaggtc agattagggc ggggagaaga
 1994

gcattccggca tgagggtga gatgcgcaa gagtgtgctc gggagtggcc cctggcacct
 2054

gggtgctctg gctggagagg aaaagccagt tccctacgag gagtgttccc aatgctttgt
 2114

ccatgatgct cttgttattt tattgccttt agaaactgag tcctgttctt gttacggcag
 2174

- 19 -

tcacactgct gggaagtggc ttaatagtaa tatcaataaa tagatgagtc ctggttagaaa
2234

aa

2236

<210> 8

<211> 448

<212> PRT

<213> Homo sapiens

<400> 8

Met	Leu	Thr	Phe	Met	Ala	Ser	Asp	Ser	Glu	Glu	Glu	Val	Cys	Asp	Glu
1				5					10					15	
Arg	Thr	Ser	Leu	Met	Ser	Ala	Glu	Ser	Pro	Thr	Pro	Arg	Ser	Cys	Gln
			20					25					30		
Glu	Gly	Arg	Gln	Gly	Pro	Glu	Asp	Gly	Glu	Asn	Thr	Ala	Gln	Trp	Arg
		35					40					45			
Ser	Gln	Glu	Asn	Glu	Glu	Asp	Gly	Glu	Glu	Asp	Pro	Asp	Arg	Tyr	Val
	50					55				60					
Cys	Ser	Gly	Val	Pro	Gly	Arg	Pro	Pro	Gly	Leu	Glu	Glu	Glu	Leu	Thr
65					70					75					80
Leu	Lys	Tyr	Gly	Ala	Lys	His	Val	Ile	Met	Leu	Phe	Val	Pro	Val	Thr
				85					90					95	
Leu	Cys	Met	Ile	Val	Val	Val	Ala	Thr	Ile	Lys	Ser	Val	Arg	Phe	Tyr
			100					105					110		
Thr	Glu	Lys	Asn	Gly	Gln	Leu	Ile	Tyr	Thr	Thr	Phe	Thr	Glu	Asp	Thr
		115					120						125		
Pro	Ser	Val	Gly	Gln	Arg	Leu	Leu	Asn	Ser	Val	Leu	Asn	Thr	Leu	Ile
	130					135					140				
Met	Ile	Ser	Val	Ile	Val	Val	Met	Thr	Ile	Phe	Leu	Val	Val	Leu	Tyr
145					150					155					160
Lys	Tyr	Arg	Cys	Tyr	Lys	Phe	Ile	His	Gly	Trp	Leu	Ile	Met	Ser	Ser
			165						170				175		
Leu	Met	Leu	Leu	Phe	Leu	Phe	Thr	Tyr	Ile	Tyr	Leu	Gly	Glu	Val	Leu
		180						185					190		
Lys	Thr	Tyr	Asn	Val	Ala	Met	Asp	Tyr	Pro	Thr	Leu	Leu	Leu	Thr	Val
		195					200					205			
Trp	Asn	Phe	Gly	Ala	Val	Gly	Met	Val	Cys	Ile	His	Trp	Lys	Gly	Pro
	210					215					220				
Leu	Val	Leu	Gln	Gln	Ala	Tyr	Leu	Ile	Met	Ile	Ser	Ala	Leu	Met	Ala
225					230					235					240
Leu	Val	Phe	Ile	Lys	Tyr	Leu	Pro	Glu	Trp	Ser	Ala	Trp	Val	Ile	Leu
			245						250					255	
Gly	Ala	Ile	Ser	Val	Tyr	Asp	Leu	Val	Ala	Val	Leu	Cys	Pro	Lys	Gly
		260					265						270		
Pro	Leu	Arg	Met	Leu	Val	Glu	Thr	Ala	Gln	Glu	Arg	Asn	Glu	Pro	Ile
		275					280					285			
Phe	Pro	Ala	Leu	Ile	Tyr	Ser	Ser	Ala	Met	Val	Trp	Thr	Val	Gly	Met
	290					295					300				
Ala	Lys	Leu	Asp	Pro	Ser	Ser	Gln	Gly	Ala	Leu	Gln	Leu	Pro	Tyr	Asp
305					310					315				320	
Pro	Glu	Met	Glu	Glu	Asp	Ser	Tyr	Asp	Ser	Phe	Gly	Glu	Pro	Ser	Tyr

- 20 -

				325					330				335				
Pro	Glu	Val	Phe	Glu	Pro	Pro	Leu	Thr	Gly	Tyr	Pro	Gly	Glu	Glu	Leu		
				340					345				350				
Glu	Glu	Glu	Glu	Glu	Arg	Gly	Val	Lys	Leu	Gly	Leu	Gly	Asp	Phe	Ile		
				355				360				365					
Phe	Tyr	Ser	Val	Leu	Val	Gly	Lys	Ala	Ala	Ala	Thr	Gly	Ser	Gly	Asp		
				370			375				380						
Trp	Asn	Thr	Thr	Leu	Ala	Cys	Phe	Val	Ala	Ile	Leu	Ile	Gly	Leu	Cys		
						390					395				400		
Leu	Thr	Leu	Leu	Leu	Ala	Val	Phe	Lys	Lys	Ala	Leu	Pro	Ala	Leu			
				405				410					415				
Pro	Ile	Ser	Ile	Thr	Phe	Gly	Leu	Ile	Phe	Tyr	Phe	Ser	Thr	Asp	Asn		
				420				425					430				
Leu	Val	Arg	Pro	Phe	Met	Asp	Thr	Leu	Ala	Ser	His	Gln	Leu	Tyr	Ile		
				435			440						445				

<210> 9

<211> 14896

<212> DNA

<213> Homo sapiens

<220>

<221> CDS

<222> (467)...(14101)

<223> nucleotide sequence encoding Human low density
lipoprotein receptor-related protein (LRP)

<400> 9

cagcgggtgcg agctccaggc ccatgcactg aggaggcgga aacaagggga gccccagag
60
ctccatcaag cccctccaa aggetcccct acccggtcca cgccccccac cccctccc
120
cgctcctcc caattgtgca tttttgcagc cggaggcggc tccgagatgg ggctgtgagc
180
ttcgccggg gaggggaaa gagcagcgag gagtgaagcg ggggggtggg gtgaagggtt
240
tggatttcgg ggcagggggc gcaccccggt cagcaggccc tcccaaggg gctcggaact
300
ctacctcttc acccacgccc ctggtgcgct ttgccgaagg aaagaataag aacagagaag
360
gaggaggggg aaaggaggaa aagggggacc cccaactgg ggggggtgaa ggagagaagt
420
agcaggacca gaggggaagg ggctgctgct tgcacagcc cacacc atg ctg acc
475

Met Leu Thr

1

ccg ccg ttg ctc ctg ctg ctg ccc ctg ctc tca gct ctg gtc gcg gcg
523

Pro Pro Leu Leu Leu Leu Leu Pro Leu Leu Ser Ala Leu Val Ala Ala

5

10

15

- 21 -

gct atc gac gcc cct aag act tgc agc ccc aag cag ttt gcc tgc aga
 571
 Ala Ile Asp Ala Pro Lys Thr Cys Ser Pro Lys Gln Phe Ala Cys Arg
 20 25 30 35
 gat caa ata acc tgt atc tca aag ggc tgg cgg tgc gac ggt gag agg
 619
 Asp Gln Ile Thr Cys Ile Ser Lys Gly Trp Arg Cys Asp Gly Glu Arg
 40 45 50
 gac tgc cca gac gga tct gac gag gcc cct gag att tgt cca cag agt
 667
 Asp Cys Pro Asp Gly Ser Asp Glu Ala Pro Glu Ile Cys Pro Gln Ser
 55 60 65
 aag gcc cag cga tgc cag cca aac gag cat aac tgc ctg ggt act gag
 715
 Lys Ala Gln Arg Cys Gln Pro Asn Glu His Asn Cys Leu Gly Thr Glu
 70 75 80
 ctg tgt gtt ccc atg tcc cgc ctc tgc aat ggg gtc cag gac tgc atg
 763
 Leu Cys Val Pro Met Ser Arg Leu Cys Asn Gly Val Gln Asp Cys Met
 85 90 95
 gac ggc tca gat gag ggg ccc cac tgc cga gag ctc caa ggc aac tgc
 811
 Asp Gly Ser Asp Glu Gly Pro His Cys Arg Glu Leu Gln Gly Asn Cys
 100 105 110 115
 tct cgc ctg ggc tgc cag cac cat tgt gtc ccc aca ctc gat ggg ccc
 859
 Ser Arg Leu Gly Cys Gln His His Cys Val Pro Thr Leu Asp Gly Pro
 120 125 130
 acc tgc tac tgc aac agc agc ttt cag ctt cag gca gat ggc aag acc
 907
 Thr Cys Tyr Cys Asn Ser Ser Phe Gln Leu Gln Ala Asp Gly Lys Thr
 135 140 145
 tgc aaa gat ttt gat gag tgc tca gtg tac ggc acc tgc agc cag cta
 955
 Cys Lys Asp Phe Asp Glu Cys Ser Val Tyr Gly Thr Cys Ser Gln Leu
 150 155 160
 tgc acc aac aca gac ggc tcc ttc ata tgt ggc tgt gtt gaa gga tac
 1003
 Cys Thr Asn Thr Asp Gly Ser Phe Ile Cys Gly Cys Val Glu Gly Tyr
 165 170 175
 ctc ctg cag ccg gat aac cgc tcc tgc aag gcc aag aac gag cca gta
 1051

- 22 -

Leu Leu Gln Pro Asp Asn Arg Ser Cys Lys Ala Lys Asn Glu Pro Val
 180 185 190 195
 gac cgg ccc cct gtg ctg ttg ata gcc aac tcc cag aac atc ttg gcc
 1099
 Asp Arg Pro Pro Val Leu Leu Ile Ala Asn Ser Gln Asn Ile Leu Ala
 200 205 210
 acg tac ctg agt ggg gcc cag gtg tct acc atc aca cct acg agc acg
 1147
 Thr Tyr Leu Ser Gly Ala Gln Val Ser Thr Ile Thr Pro Thr Ser Thr
 215 220 225
 cgg cag acc aca gcc atg gac ttc agc tat gcc aac gag acc gta tgc
 1195
 Arg Gln Thr Thr Ala Met Asp Phe Ser Tyr Ala Asn Glu Thr Val Cys
 230 235 240
 tgg gtg cat gtt ggg gac agt gct gct cag acg cag ctc aag tgt gcc
 1243
 Trp Val His Val Gly Asp Ser Ala Ala Gln Thr Gln Leu Lys Cys Ala
 245 250 255
 cgc atg cct ggc cta aag ggc ttc gtg gat gag cac acc atc aac atc
 1291
 Arg Met Pro Gly Leu Lys Gly Phe Val Asp Glu His Thr Ile Asn Ile
 260 265 270 275
 tcc ctc agt ctg cac cac gtg gaa cag atg gcc atc gac tgg ctg aca
 1339
 Ser Leu Ser Leu His His Val Glu Gln Met Ala Ile Asp Trp Leu Thr
 280 285 290
 ggc aac ttc tac ttt gtg gat gac atc gat gat agg atc ttt gtc tgc
 1387
 Gly Asn Phe Tyr Phe Val Asp Asp Ile Asp Asp Arg Ile Phe Val Cys
 295 300 305
 aac aga aat ggg gac aca tgt gtc aca ttg cta gac ctg gaa ctc tac
 1435
 Asn Arg Asn Gly Asp Thr Cys Val Thr Leu Leu Asp Leu Glu Leu Tyr
 310 315 320
 aac ccc aag ggc att gcc ctg gac cct gcc atg ggg aag gtg ttt ttc
 1483
 Asn Pro Lys Gly Ile Ala Leu Asp Pro Ala Met Gly Lys Val Phe Phe
 325 330 335
 act gac tat ggg cag atc cca aag gtg gaa cgc tgt gac atg gat ggg
 1531
 Thr Asp Tyr Gly Gln Ile Pro Lys Val Glu Arg Cys Asp Met Asp Gly
 340 345 350 355

- 23 -

cag aac cgc acc aag ctc gtc gac agc aag att gtg ttt cct cat ggc
 1579
 Gln Asn Arg Thr Lys Leu Val Asp Ser Lys Ile Val Phe Pro His Gly
 360 365 370

atc acg ctg gac ctg gtc agc cgc ctt gtc tac tgg gca gat gcc tat
 1627
 Ile Thr Leu Asp Leu Val Ser Arg Leu Val Tyr Trp Ala Asp Ala Tyr
 375 380 385

ctg gac tat att gaa gtg gtg gac tat gag ggc aag ggc cgc cag acc
 1675
 Leu Asp Tyr Ile Glu Val Val Asp Tyr Glu Gly Lys Gly Arg Gln Thr
 390 395 400

atc atc cag ggc atc ctg att gag cac ctg tac ggc ctg act gtg ttt
 1723
 Ile Ile Gln Gly Ile Leu Ile Glu His Leu Tyr Gly Leu Thr Val Phe
 405 410 415

gag aat tat ctc tat gcc acc aac tcg gac aat gcc aat gcc cag cag
 1771
 Glu Asn Tyr Leu Tyr Ala Thr Asn Ser Asp Asn Ala Asn Ala Gln Gln
 420 425 430 435

aag acg agt gtg atc cgt gtg aac cgc ttt aac agc acc gag tac cag
 1819
 Lys Thr Ser Val Ile Arg Val Asn Arg Phe Asn Ser Thr Glu Tyr Gln
 440 445 450

gtt gtc acc cgg gtg gac aag ggt ggt gcc ctc cac atc tac cac cag
 1867
 Val Val Thr Arg Val Asp Lys Gly Gly Ala Leu His Ile Tyr His Gln
 455 460 465

agg cgt cag ccc cga gtg agg agc cat gcc tgt gaa aac gac cag tat
 1915
 Arg Arg Gln Pro Arg Val Arg Ser His Ala Cys Glu Asn Asp Gln Tyr
 470 475 480

ggg aag ccg ggt ggc tgc tct gac atc tgc ctg ctg gcc aac agc cac
 1963
 Gly Lys Pro Gly Gly Cys Ser Asp Ile Cys Leu Leu Ala Asn Ser His
 485 490 495

aag gcg cgg acc tgc cgc tgc cgt tcc ggc ttc agc ctg ggc agt gac
 2011
 Lys Ala Arg Thr Cys Arg Cys Arg Ser Gly Phe Ser Leu Gly Ser Asp
 500 505 510 515

- 24 -

ggg aag tca tgc aag aag ccg gag cat gag ctg ttc ctc gtg tat ggc
 2059
 Gly Lys Ser Cys Lys Lys Pro Glu His Glu Leu Phe Leu Val Tyr Gly
 520 525 530

aag ggc cgg cca ggc atc atc cgg ggc atg gat atg ggg gcc aag gtc
 2107
 Lys Gly Arg Pro Gly Ile Ile Arg Gly Met Asp Met Gly Ala Lys Val
 535 540 545

ccg gat gag cac atg atc ccc att gaa aac ctc atg aac ccc cga gcc
 2155
 Pro Asp Glu His Met Ile Pro Ile Glu Asn Leu Met Asn Pro Arg Ala
 550 555 560

ctg gac ttc cac gct gag acc ggc ttc atc tac ttt gcc gac acc acc
 2203
 Leu Asp Phe His Ala Glu Thr Gly Phe Ile Tyr Phe Ala Asp Thr Thr
 565 570 575

agc tac ctc att ggc cgc cag aag att gat ggc act gag cgg gag acc
 2251
 Ser Tyr Leu Ile Gly Arg Gln Lys Ile Asp Gly Thr Glu Arg Glu Thr
 580 585 590 595

atc ctg aag gac ggc atc cac aat gtg gag ggt gtg gcc gtg gac tgg
 2299
 Ile Leu Lys Asp Gly Ile His Asn Val Glu Gly Val Ala Val Asp Trp
 600 605 610

atg gga gac aat ctg tac tgg acg gac gat ggg ccc aaa aag aca atc
 2347
 Met Gly Asp Asn Leu Tyr Trp Thr Asp Asp Gly Pro Lys Lys Thr Ile
 615 620 625

agc gtg gcc agg ctg gag aaa gct gct cag acc cgc aag act tta atc
 2395
 Ser Val Ala Arg Leu Glu Lys Ala Ala Gln Thr Arg Lys Thr Leu Ile
 630 635 640

gag ggc aaa atg aca cac ccc agg gct att gtg gtg gat cca ctc aat
 2443
 Glu Gly Lys Met Thr His Pro Arg Ala Ile Val Val Asp Pro Leu Asn
 645 650 655

ggg tgg atg tac tgg aca gac tgg gag gag gac ccc aag gac agt cgg
 2491
 Gly Trp Met Tyr Trp Thr Asp Trp Glu Glu Asp Pro Lys Asp Ser Arg
 660 665 670 675

cgt ggg cgg ctg gag agg gcg tgg atg gat ggc tca cac cga gac atc
 2539

- 25 -

Arg Gly Arg Leu Glu Arg Ala Trp Met Asp Gly Ser His Arg Asp Ile
 680 685 690
 ttt gtc acc tcc aag aca gtg ctt tgg ccc aat ggg cta agc ctg gac
 2587
 Phe Val Thr Ser Lys Thr Val Leu Trp Pro Asn Gly Leu Ser Leu Asp
 695 700 705
 atc ccg gct ggg cgc ctc tac tgg gtg gat gcc ttc tac gac cgc atc
 2635
 Ile Pro Ala Gly Arg Leu Tyr Trp Val Asp Ala Phe Tyr Asp Arg Ile
 710 715 720
 gag acg ata ctg ctc aat ggc aca gac cgg aag att gtg tat gaa ggt
 2683
 Glu Thr Ile Leu Leu Asn Gly Thr Asp Arg Lys Ile Val Tyr Glu Gly
 725 730 735
 cct gag ctg aac cac gcc ttt ggc ctg tgt cac cat ggc aac tac ctc
 2731
 Pro Glu Leu Asn His Ala Phe Gly Leu Cys His His Gly Asn Tyr Leu
 740 745 750 755
 ttc tgg act gag tat cgg agt ggc agt gtc tac cgc ttg gaa cgg ggt
 2779
 Phe Trp Thr Glu Tyr Arg Ser Gly Ser Val Tyr Arg Leu Glu Arg Gly
 760 765 770
 gta gga ggc gca ccc ccc act gtg acc ctt ctg cgc agt gag cgg ccc
 2827
 Val Gly Gly Ala Pro Pro Thr Val Thr Leu Leu Arg Ser Glu Arg Pro
 775 780 785
 ccc atc ttt gag atc cga atg tat gat gcc cag cag cag caa gtt ggc
 2875
 Pro Ile Phe Glu Ile Arg Met Tyr Asp Ala Gln Gln Gln Gln Val Gly
 790 795 800
 acc aac aaa tgc cgg gtg aac aat ggc ggc tgc agc agc ctg tgc ttg
 2923
 Thr Asn Lys Cys Arg Val Asn Asn Gly Gly Cys Ser Ser Leu Cys Leu
 805 810 815
 gcc acc cct ggg agc cgc cag tgc gcc tgt gct gag gac cag gtg ttg
 2971
 Ala Thr Pro Gly Ser Arg Gln Cys Ala Cys Ala Glu Asp Gln Val Leu
 820 825 830 835
 gac gca gac ggc gtc act tgc ttg gcg aac cca tcc tac gtg cct cca
 3019
 Asp Ala Asp Gly Val Thr Cys Leu Ala Asn Pro Ser Tyr Val Pro Pro
 840 845 850

- 26 -

ccc cag tgc cag cca ggc gag ttt gcc tgt gcc aac agc cgc tgc atc
3067

Pro Gln Cys Gln Pro Gly Glu Phe Ala Cys Ala Asn Ser Arg Cys Ile
855 860 865

cag gag cgc tgg aag tgt gac gga gac aac gat tgc ctg gac aac agt
3115

Gln Glu Arg Trp Lys Cys Asp Gly Asp Asn Asp Cys Leu Asp Asn Ser
870 875 880

gat gag gcc cca gcc ctc tgc cat cag cac acc tgc ccc tcg gac cga
3163

Asp Glu Ala Pro Ala Leu Cys His Gln His Thr Cys Pro Ser Asp Arg
885 890 895

ttc aag tgc gag aac aac cgg tgc atc ccc aac cgc tgg ctc tgc gac
3211

Phe Lys Cys Glu Asn Asn Arg Cys Ile Pro Asn Arg Trp Leu Cys Asp
900 905 910 915

ggg gac aat gac tgt ggg aac agt gaa gat gag tcc aat gcc act tgt
3259

Gly Asp Asn Asp Cys Gly Asn Ser Glu Asp Glu Ser Asn Ala Thr Cys
920 925 930

tca gcc cgc acc tgc ccc ccc aac cag ttc tcc tgt gcc agt ggc cgc
3307

Ser Ala Arg Thr Cys Pro Pro Asn Gln Phe Ser Cys Ala Ser Gly Arg
935 940 945

tgc atc ccc atc tcc tgg acg tgt gat ctg gat gac gac tgt ggg gac
3355

Cys Ile Pro Ile Ser Trp Thr Cys Asp Leu Asp Asp Asp Cys Gly Asp
950 955 960

cgc tct gat gag tct gct tcg tgt gcc tat ccc acc tgc ttc ccc ctg
3403

Arg Ser Asp Glu Ser Ala Ser Cys Ala Tyr Pro Thr Cys Phe Pro Leu
965 970 975

act cag ttt acc tgc aac aat ggc aga tgt atc aac atc aac tgg aga
3451

Thr Gln Phe Thr Cys Asn Asn Gly Arg Cys Ile Asn Ile Asn Trp Arg
980 985 990 995

tgc gac aat gac aat gac tgt ggg gac aac agt gac gaa gcc ggc tgc
3499

Cys Asp Asn Asp Asn Asp Cys Gly Asp Asn Ser Asp Glu Ala Gly Cys
1000 1005 1010

- 27 -

agc cac tcc tgt tct agc acc cag ttc aag tgc aac agc ggg cgt tgc
3547

Ser His Ser Cys Ser Ser Thr Gln Phe Lys Cys Asn Ser Gly Arg Cys
1015 1020 1025

atc ccc gag cac tgg acc tgc gat ggg gac aat gac tgc gga gac tac
3595

Ile Pro Glu His Trp Thr Cys Asp Gly Asp Asn Asp Cys Gly Asp Tyr
1030 1035 1040

agt gat gag aca cac gcc aac tgc acc aac cag gcc acg agg ccc cct
3643

Ser Asp Glu Thr His Ala Asn Cys Thr Asn Gln Ala Thr Arg Pro Pro
1045 1050 1055

ggc ggc tgc cac act gat gag ttc cag tgc cgg ctg gat gga cta tgc
3691

Gly Gly Cys His Thr Asp Glu Phe Gln Cys Arg Leu Asp Gly Leu Cys
1060 1065 1070 1075

atc ccc ctg cgg tgg cgc tgc gat ggg gac act gac tgc atg gac tcc
3739

Ile Pro Leu Arg Trp Arg Cys Asp Gly Asp Thr Asp Cys Met Asp Ser
1080 1085 1090

agc gat gag aag agc tgt gag gga gtg acc cac gtc tgc gat ccc agt
3787

Ser Asp Glu Lys Ser Cys Glu Gly Val Thr His Val Cys Asp Pro Ser
1095 1100 1105

gtc aag ttt ggc tgc aag gac tca gct cgg tgc atc agc aaa gcg tgg
3835

Val Lys Phe Gly Cys Lys Asp Ser Ala Arg Cys Ile Ser Lys Ala Trp
1110 1115 1120

gtg tgt gat ggc gac aat gac tgt gag gat aac tcg gac gag gag aac
3883

Val Cys Asp Gly Asp Asn Asp Cys Glu Asp Asn Ser Asp Glu Glu Asn
1125 1130 1135

tgc gag tcc ctg gcc tgc agg cca ccc tcg cac cct tgt gcc aac aac
3931

Cys Glu Ser Leu Ala Cys Arg Pro Pro Ser His Pro Cys Ala Asn Asn
1140 1145 1150 1155

acc tca gtc tgc ctg ccc cct gac aag ctg tgt gat ggc aac gac gac
3979

Thr Ser Val Cys Leu Pro Pro Asp Lys Leu Cys Asp Gly Asn Asp Asp
1160 1165 1170

tgt ggc gac ggc tca gat gag ggc gag ctc tgc gac cag tgc tct ctg
4027

- 28 -

Cys Gly Asp Gly Ser Asp Glu Gly Glu Leu Cys Asp Gln Cys Ser Leu
 1175 1180 1185
 aat aac ggt ggc tgc agc cac aac tgc tca gtg gca cct ggc gaa ggc
 4075
 Asn Asn Gly Gly Cys Ser His Asn Cys Ser Val Ala Pro Gly Glu Gly
 1190 1195 1200
 att gtg tgt tcc tgc cct ctg ggc atg gag ctg ggg ccc gac aac cac
 4123
 Ile Val Cys Ser Cys Pro Leu Gly Met Glu Leu Gly Pro Asp Asn His
 1205 1210 1215
 acc tgc cag atc cag agc tac tgt gcc aag cat ctc aaa tgc agc caa
 4171
 Thr Cys Gln Ile Gln Ser Tyr Cys Ala Lys His Leu Lys Cys Ser Gln
 1220 1225 1230 1235
 aag tgc gac cag aac aag ttc agc gtg aag tgc tcc tgc tac gag ggc
 4219
 Lys Cys Asp Gln Asn Lys Phe Ser Val Lys Cys Ser Cys Tyr Glu Gly
 1240 1245 1250
 tgg gtc ctg gaa cct gac ggc gag agc tgc cgc agc ctg gac ccc ttc
 4267
 Trp Val Leu Glu Pro Asp Gly Glu Ser Cys Arg Ser Leu Asp Pro Phe
 1255 1260 1265
 aag ccg ttc atc att ttc tcc aac cgc cat gaa atc cgg cgc atc gat
 4315
 Lys Pro Phe Ile Ile Phe Ser Asn Arg His Glu Ile Arg Arg Ile Asp
 1270 1275 1280
 ctt cac aaa gga gac tac agc gtc ctg gtg ccc ggc ctg cgc aac acc
 4363
 Leu His Lys Gly Asp Tyr Ser Val Leu Val Pro Gly Leu Arg Asn Thr
 1285 1290 1295
 atc gcc ctg gac ttc cac ctc agc cag agc gcc ctc tac tgg acc gac
 4411
 Ile Ala Leu Asp Phe His Leu Ser Gln Ser Ala Leu Tyr Trp Thr Asp
 1300 1305 1310 1315
 gtg gtg gag gac aag atc tac cgc ggg aag ctg ctg gac aac gga gcc
 4459
 Val Val Glu Asp Lys Ile Tyr Arg Gly Lys Leu Leu Asp Asn Gly Ala
 1320 1325 1330
 ctg act agt ttc gag gtg gtg att cag tat ggc ctg gcc aca ccc gag
 4507
 Leu Thr Ser Phe Glu Val Val Ile Gln Tyr Gly Leu Ala Thr Pro Glu
 1335 1340 1345

- 29 -

ggc ctg gct gta gac tgg att gca ggc aac atc tac tgg gtg gag agt
4555

Gly Leu Ala Val Asp Trp Ile Ala Gly Asn Ile Tyr Trp Val Glu Ser
1350 1355 1360

aac ctg gat cag atc gag gtg gcc aag ctg gat ggg acc ctc cgg acc
4603

Asn Leu Asp Gln Ile Glu Val Ala Lys Leu Asp Gly Thr Leu Arg Thr
1365 1370 1375

acc ctg ctg gcc ggt gac att gag cac cca agg gca atc gca ctg gat
4651

Thr Leu Leu Ala Gly Asp Ile Glu His Pro Arg Ala Ile Ala Leu Asp
1380 1385 1390 1395

ccc cgg gat ggg atc ctg ttt tgg aca gac tgg gat gcc agc ctg ccc
4699

Pro Arg Asp Gly Ile Leu Phe Trp Thr Asp Trp Asp Ala Ser Leu Pro
1400 1405 1410

cgc att gag gca gcc tcc atg agt ggg gct ggg cgc cgc acc gtg cac
4747

Arg Ile Glu Ala Ala Ser Met Ser Gly Ala Gly Arg Arg Thr Val His
1415 1420 1425

cgg gag acc ggc tct ggg ggc tgg ccc aac ggg ctc acc gtg gac tac
4795

Arg Glu Thr Gly Ser Gly Gly Trp Pro Asn Gly Leu Thr Val Asp Tyr
1430 1435 1440

ctg gag aag cgc atc ctt tgg att gac gcc agg tca gat gcc att tac
4843

Leu Glu Lys Arg Ile Leu Trp Ile Asp Ala Arg Ser Asp Ala Ile Tyr
1445 1450 1455

tca gcc cgt tac gac ggc tct ggc cac atg gag gtg ctt cgg gga cac
4891

Ser Ala Arg Tyr Asp Gly Ser Gly His Met Glu Val Leu Arg Gly His
1460 1465 1470 1475

gag ttc ctg tcg cac ccg ttt gca gtg acg ctg tac ggg ggg gag gtc
4939

Glu Phe Leu Ser His Pro Phe Ala Val Thr Leu Tyr Gly Gly Glu Val
1480 1485 1490

tac tgg act gac tgg cga aca aac aca ctg gct aag gcc aac aag tgg
4987

Tyr Trp Thr Asp Trp Arg Thr Asn Thr Leu Ala Lys Ala Asn Lys Trp
1495 1500 1505

- 30 -

acc ggc cac aat gtc acc gtg gta cag agg acc aac acc cag ccc ttt
5035

Thr Gly His Asn Val Thr Val Val Gln Arg Thr Asn Thr Gln Pro Phe
1510 1515 1520

gac ctg cag gtg tac cac ccc tcc cgc cag ccc atg gct ccc aat ccc
5083

Asp Leu Gln Val Tyr His Pro Ser Arg Gln Pro Met Ala Pro Asn Pro
1525 1530 1535

tgt gag gcc aat ggg ggc cag ggc ccc tgc tcc cac ctg tgt ctc atc
5131

Cys Glu Ala Asn Gly Gly Gln Gly Pro Cys Ser His Leu Cys Leu Ile
1540 1545 1550 1555

aac tac aac cgg acc gtg tcc tgc gcc tgc ccc cac ctc atg aag ctc
5179

Asn Tyr Asn Arg Thr Val Ser Cys Ala Cys Pro His Leu Met Lys Leu
1560 1565 1570

cac aag gac aac acc acc tgc tat gag ttt aag aag ttc ctg ctg tac
5227

His Lys Asp Asn Thr Thr Cys Tyr Glu Phe Lys Lys Phe Leu Leu Tyr
1575 1580 1585

gca cgt cag atg gag atc cga ggt gtg gac ctg gat gct ccc tac tac
5275

Ala Arg Gln Met Glu Ile Arg Gly Val Asp Leu Asp Ala Pro Tyr Tyr
1590 1595 1600

aac tac atc atc tcc ttc acg gtg ccc gac atc gac aac gtc aca gtg
5323

Asn Tyr Ile Ile Ser Phe Thr Val Pro Asp Ile Asp Asn Val Thr Val
1605 1610 1615

cta gac tac gat gcc cgc gag cag cgt gtg tac tgg tct gac gtg cgg
5371

Leu Asp Tyr Asp Ala Arg Glu Gln Arg Val Tyr Trp Ser Asp Val Arg
1620 1625 1630 1635

aca cag gcc atc aag cgg gcc ttc atc aac ggc aca ggc gtg gag aca
5419

Thr Gln Ala Ile Lys Arg Ala Phe Ile Asn Gly Thr Gly Val Glu Thr
1640 1645 1650

gtc gtc tct gca gac ttg cca aat gcc cac ggg ctg gct gtg gac tgg
5467

Val Val Ser Ala Asp Leu Pro Asn Ala His Gly Leu Ala Val Asp Trp
1655 1660 1665

gtc tcc cga aac ctg ttc tgg aca agc tat gac acc aat aag aag cag
5515

- 31 -

Val Ser Arg Asn Leu Phe Trp Thr Ser Tyr Asp Thr Asn Lys Lys Gln
 1670 1675 1680
 atc aat gtg gcc cgg ctg gat ggc tcc ttc aag aac gca gtg gtg cag
 5563
 Ile Asn Val Ala Arg Leu Asp Gly Ser Phe Lys Asn Ala Val Val Gln
 1685 1690 1695
 ggc ctg gag cag ccc cat ggc ctt gtc gtc cac cct ctg cgt ggg aag
 5611
 Gly Leu Glu Gln Pro His Gly Leu Val Val His Pro Leu Arg Gly Lys
 1700 1705 1710 1715
 ctc tac tgg acc gat ggt gac aac atc agc atg gcc aac atg gat ggc
 5659
 Leu Tyr Trp Thr Asp Gly Asp Asn Ile Ser Met Ala Asn Met Asp Gly
 1720 1725 1730
 agc aat cgc acc ctg ctc ttc agt ggc cag aag ggc ccc gtg ggc ctg
 5707
 Ser Asn Arg Thr Leu Leu Phe Ser Gly Gln Lys Gly Pro Val Gly Leu
 1735 1740 1745
 gct att gac ttc cct gaa agc aaa ctc tac tgg atc agc tcc ggg aac
 5755
 Ala Ile Asp Phe Pro Glu Ser Lys Leu Tyr Trp Ile Ser Ser Gly Asn
 1750 1755 1760
 cat acc atc aac cgc tgc aac ctg gat ggg agt ggg ctg gag gtc atc
 5803
 His Thr Ile Asn Arg Cys Asn Leu Asp Gly Ser Gly Leu Glu Val Ile
 1765 1770 1775
 gat gcc atg cgg agc cag ctg ggc aag gcc acc gcc ctg gcc atc atg
 5851
 Asp Ala Met Arg Ser Gln Leu Gly Lys Ala Thr Ala Leu Ala Ile Met
 1780 1785 1790 1795
 ggg gac aag ctg tgg tgg gct gat cag gtg tcg gaa aag atg ggc aca
 5899
 Gly Asp Lys Leu Trp Trp Ala Asp Gln Val Ser Glu Lys Met Gly Thr
 1800 1805 1810
 tgc agc aag gct gac ggc tcg ggc tcc gtg gtc ctt cgg aac agc acc
 5947
 Cys Ser Lys Ala Asp Gly Ser Gly Ser Val Val Leu Arg Asn Ser Thr
 1815 1820 1825
 acc ctg gtg atg cac atg aag gtc tat gac gag agc atc cag ctg gac
 5995
 Thr Leu Val Met His Met Lys Val Tyr Asp Glu Ser Ile Gln Leu Asp
 1830 1835 1840

- 32 -

cat aag ggc acc aac ccc tgc agt gtc aac aac ggt gac tgc tcc cag
6043

His Lys Gly Thr Asn Pro Cys Ser Val Asn Asn Gly Asp Cys Ser Gln
1845 1850 1855

ctc tgc ctg ccc acg tca gag acg acc cgc tcc tgc atg tgc aca gcc
6091

Leu Cys Leu Pro Thr Ser Glu Thr Thr Arg Ser Cys Met Cys Thr Ala
1860 1865 1870 1875

ggc tat agc ctc cgg agt ggc cag cag gcc tgc gag ggc gta ggt tcc
6139

Gly Tyr Ser Leu Arg Ser Gly Gln Gln Ala Cys Glu Gly Val Gly Ser
1880 1885 1890

ttt ctc ctg tac tct gtg cat gag gga atc agg gga att ccc ctg gat
6187

Phe Leu Leu Tyr Ser Val His Glu Gly Ile Arg Gly Ile Pro Leu Asp
1895 1900 1905

ccc aat gac aag tca gat gcc ctg gtc cca gtg tcc ggg acc tcg ctg
6235

Pro Asn Asp Lys Ser Asp Ala Leu Val Pro Val Ser Gly Thr Ser Leu
1910 1915 1920

gct gtc ggc atc gac ttc cac gct gaa aat gac acc atc tac tgg gtg
6283

Ala Val Gly Ile Asp Phe His Ala Glu Asn Asp Thr Ile Tyr Trp Val
1925 1930 1935

gac atg ggc ctg agc acg atc agc cgg gcc aag cgg gac cag acg tgg
6331

Asp Met Gly Leu Ser Thr Ile Ser Arg Ala Lys Arg Asp Gln Thr Trp
1940 1945 1950 1955

cgt gaa gac gtg gtg acc aat ggc att ggc cgt gtg gag ggc att gca
6379

Arg Glu Asp Val Val Thr Asn Gly Ile Gly Arg Val Glu Gly Ile Ala
1960 1965 1970

gtg gac tgg atc gca ggc aac atc tac tgg aca gac cag ggc ttt gat
6427

Val Asp Trp Ile Ala Gly Asn Ile Tyr Trp Thr Asp Gln Gly Phe Asp
1975 1980 1985

gtc atc gag gtc gcc cgg ctc aat ggc tcc ttc cgc tac gtg gtg atc
6475

Val Ile Glu Val Ala Arg Leu Asn Gly Ser Phe Arg Tyr Val Val Ile
1990 1995 2000

- 33 -

tcc cag ggt cta gac aag ccc cgg gcc atc acc gtc cac ccg gag aaa
6523

Ser Gln Gly Leu Asp Lys Pro Arg Ala Ile Thr Val His Pro Glu Lys
2005 2010 2015

ggg tac ttg ttc tgg act gag tgg ggt cag tat ccg cgt att gag cgg
6571

Gly Tyr Leu Phe Trp Thr Glu Trp Gly Gln Tyr Pro Arg Ile Glu Arg
2020 2025 2030 2035

tct cgg cta gat ggc acg gag cgt gtg gtg ctg gtc aac gtc agc atc
6619

Ser Arg Leu Asp Gly Thr Glu Arg Val Val Leu Val Asn Val Ser Ile
2040 2045 2050

agc tgg ccc aac ggc atc tca gtg gac tac cag gat ggg aag ctg tac
6667

Ser Trp Pro Asn Gly Ile Ser Val Asp Tyr Gln Asp Gly Lys Leu Tyr
2055 2060 2065

tgg tgc gat gca cgg aca gac aag att gaa cgg atc gac ctg gag aca
6715

Trp Cys Asp Ala Arg Thr Asp Lys Ile Glu Arg Ile Asp Leu Glu Thr
2070 2075 2080

ggt gag aac cgc gag gtg gtt ctg tcc agc aac aac atg gac atg ttt
6763

Gly Glu Asn Arg Glu Val Val Leu Ser Ser Asn Asn Met Asp Met Phe
2085 2090 2095

tca gtg tct gtg ttt gag gat ttc atc tac tgg agt gac agg act cat
6811

Ser Val Ser Val Phe Glu Asp Phe Ile Tyr Trp Ser Asp Arg Thr His
2100 2105 2110 2115

gcc aac ggc tct atc aag cgc ggg agc aaa gac aat gcc aca gac tcc
6859

Ala Asn Gly Ser Ile Lys Arg Gly Ser Lys Asp Asn Ala Thr Asp Ser
2120 2125 2130

gtg ccc ctg cga acc ggc atc ggc gtc cag ctt aaa gac atc aaa gtc
6907

Val Pro Leu Arg Thr Gly Ile Gly Val Gln Leu Lys Asp Ile Lys Val
2135 2140 2145

ttc aac cgg gac cgg cag aaa ggc acc aac gtg tgc gcg gtg gcc aat
6955

Phe Asn Arg Asp Arg Gln Lys Gly Thr Asn Val Cys Ala Val Ala Asn
2150 2155 2160

ggc ggg tgc cag cag ctg tgc ctg tac cgg ggc cgt ggg cag cgg gcc
7003

- 34 -

Gly Gly Cys Gln Gln Leu Cys Leu Tyr Arg Gly Arg Gly Gln Arg Ala
 2165 2170 2175
 tgc gcc tgt gcc cac ggg atg ctg gct gaa gac gga gca tcg tgc cgc
 7051
 Cys Ala Cys Ala His Gly Met Leu Ala Glu Asp Gly Ala Ser Cys Arg
 2180 2185 2190 2195
 gag tat gcc ggc tac ctg ctc tac tca gag cgc acc att ctc aag agt
 7099
 Glu Tyr Ala Gly Tyr Leu Leu Tyr Ser Glu Arg Thr Ile Leu Lys Ser
 2200 2205 2210
 atc cac ctg tcg gat gag cgc aac ctc aat gcg ccc gtg cag ccc ttc
 7147
 Ile His Leu Ser Asp Glu Arg Asn Leu Asn Ala Pro Val Gln Pro Phe
 2215 2220 2225
 gag gac cct gag cac atg aag aac gtc atc gcc ctg gcc ttt gac tac
 7195
 Glu Asp Pro Glu His Met Lys Asn Val Ile Ala Leu Ala Phe Asp Tyr
 2230 2235 2240
 cgg gca ggc acc tct ccg ggc acc ccc aat cgc atc ttc ttc agc gac
 7243
 Arg Ala Gly Thr Ser Pro Gly Thr Pro Asn Arg Ile Phe Phe Ser Asp
 2245 2250 2255
 atc cac ttt ggg aac atc caa cag atc aac gac gat ggc tcc agg agg
 7291
 Ile His Phe Gly Asn Ile Gln Gln Ile Asn Asp Asp Gly Ser Arg Arg
 2260 2265 2270 2275
 atc acc att gtg gaa aac gtg ggc tcc gtg gaa ggc ctg gcc tat cac
 7339
 Ile Thr Ile Val Glu Asn Val Gly Ser Val Glu Gly Leu Ala Tyr His
 2280 2285 2290
 cgt ggc tgg gac act ctc tat tgg aca agc tac acg aca tcc acc atc
 7387
 Arg Gly Trp Asp Thr Leu Tyr Trp Thr Ser Tyr Thr Thr Ser Thr Ile
 2295 2300 2305
 acg cgc cac aca gtg gac cag acc cgc cca ggg gcc ttc gag cgt gag
 7435
 Thr Arg His Thr Val Asp Gln Thr Arg Pro Gly Ala Phe Glu Arg Glu
 2310 2315 2320
 acc gtc atc act atg tct gga gat gac cac cca cgg gcc ttc gtt ttg
 7483
 Thr Val Ile Thr Met Ser Gly Asp Asp His Pro Arg Ala Phe Val Leu
 2325 2330 2335

- 35 -

gac gag tgc cag aac ctc atg ttc tgg acc aac tgg aat gag cag cat
 7531
 Asp Glu Cys Gln Asn Leu Met Phe Trp Thr Asn Trp Asn Glu Gln His
 2340 2345 2350 2355
 ccc agc atc atg cgg gcg gcg ctc tcg gga gcc aat gtc ctg acc ctt
 7579
 Pro Ser Ile Met Arg Ala Ala Leu Ser Gly Ala Asn Val Leu Thr Leu
 2360 2365 2370
 atc gag aag gac atc cgt acc ccc aat ggc ctg gcc atc gac cac cgt
 7627
 Ile Glu Lys Asp Ile Arg Thr Pro Asn Gly Leu Ala Ile Asp His Arg
 2375 2380 2385
 gcc gag aag ctc tac ttc tct gac gcc acc ctg gac aag atc gag cgg
 7675
 Ala Glu Lys Leu Tyr Phe Ser Asp Ala Thr Leu Asp Lys Ile Glu Arg
 2390 2395 2400
 tgc gag tat gac ggc tcc cac cgc tat gtg atc cta aag tca gag cct
 7723
 Cys Glu Tyr Asp Gly Ser His Arg Tyr Val Ile Leu Lys Ser Glu Pro
 2405 2410 2415
 gtc cac ccc ttc ggg ctg gcc gtg tat ggg gag cac att ttc tgg act
 7771
 Val His Pro Phe Gly Leu Ala Val Tyr Gly Glu His Ile Phe Trp Thr
 2420 2425 2430 2435
 gac tgg gtg cgg cgg gca gtg cag cgg gcc aac aag cac gtg ggc agc
 7819
 Asp Trp Val Arg Arg Ala Val Gln Arg Ala Asn Lys His Val Gly Ser
 2440 2445 2450
 aac atg aag ctg ctg cgc gtg gac atc ccc cag cag ccc atg ggc atc
 7867
 Asn Met Lys Leu Leu Arg Val Asp Ile Pro Gln Gln Pro Met Gly Ile
 2455 2460 2465
 atc gcc gtg gcc aac gac acc aac agc tgt gaa ctc tct cca tgc cga
 7915
 Ile Ala Val Ala Asn Asp Thr Asn Ser Cys Glu Leu Ser Pro Cys Arg
 2470 2475 2480
 atc aac aac ggt ggc tgc cag gac ctg tgt ctg ctc act cac cag ggc
 7963
 Ile Asn Asn Gly Gly Cys Gln Asp Leu Cys Leu Leu Thr His Gln Gly
 2485 2490 2495

- 36 -

cat gtc aac tgc tca tgc cga ggg ggc cga atc ctc cag gat gac ctc
 8011
 His Val Asn Cys Ser Cys Arg Gly Gly Arg Ile Leu Gln Asp Asp Leu
 2500 2505 2510 2515
 acc tgc cga gcg gtg aat tcc tct tgc cga gca caa gat gag ttt gag
 8059
 Thr Cys Arg Ala Val Asn Ser Ser Cys Arg Ala Gln Asp Glu Phe Glu
 2520 2525 2530
 tgt gcc aat ggc gag tgc atc aac ttc agc ctg acc tgc gac ggc gtc
 8107
 Cys Ala Asn Gly Glu Cys Ile Asn Phe Ser Leu Thr Cys Asp Gly Val
 2535 2540 2545
 ccc cac tgc aag gac aag tcc gat gag aag cca tcc tac tgc aac tcc
 8155
 Pro His Cys Lys Asp Lys Ser Asp Glu Lys Pro Ser Tyr Cys Asn Ser
 2550 2555 2560
 cgc cgc tgc aag aag act ttc cgg cag tgc agc aat ggg cgc tgt gtg
 8203
 Arg Arg Cys Lys Lys Thr Phe Arg Gln Cys Ser Asn Gly Arg Cys Val
 2565 2570 2575
 tcc aac atg ctg tgg tgc aac ggg gcc gac gac tgt ggg gat ggc tct
 8251
 Ser Asn Met Leu Trp Cys Asn Gly Ala Asp Asp Cys Gly Asp Gly Ser
 2580 2585 2590 2595
 gac gag atc cct tgc aac aag aca gcc tgt ggt gtg ggc gag ttc cgc
 8299
 Asp Glu Ile Pro Cys Asn Lys Thr Ala Cys Gly Val Gly Glu Phe Arg
 2600 2605 2610
 tgc cgg gac ggg acc tgc atc ggg aac tcc agc cgc tgc aac cag ttt
 8347
 Cys Arg Asp Gly Thr Cys Ile Gly Asn Ser Ser Arg Cys Asn Gln Phe
 2615 2620 2625
 gtg gat tgt gag gac gcc tca gat gag atg aac tgc agt gcc acc gac
 8395
 Val Asp Cys Glu Asp Ala Ser Asp Glu Met Asn Cys Ser Ala Thr Asp
 2630 2635 2640
 tgc agc agc tac ttc cgc ctg ggc gtg aag ggc gtg ctc ttc cag ccc
 8443
 Cys Ser Ser Tyr Phe Arg Leu Gly Val Lys Gly Val Leu Phe Gln Pro
 2645 2650 2655
 tgc gag cgg acc tca ctc tgc tac gca ccc agc tgg gtg tgt gat ggc
 8491

- 37 -

Cys Glu Arg Thr Ser Leu Cys Tyr Ala Pro Ser Trp Val Cys Asp Gly
 2660 2665 2670 2675
 gcc aat gac tgt ggg gac tac agt gat gag cgc gac tgc cca ggt gtg
 8539
 Ala Asn Asp Cys Gly Asp Tyr Ser Asp Glu Arg Asp Cys Pro Gly Val
 2680 2685 2690
 aaa cgc ccc aga tgc cct ctg aat tac ttc gcc tgc cct agt ggg cgc
 8587
 Lys Arg Pro Arg Cys Pro Leu Asn Tyr Phe Ala Cys Pro Ser Gly Arg
 2695 2700 2705
 tgc atc ccc atg agc tgg acg tgt gac aaa gag gat gac tgt gaa cat
 8635
 Cys Ile Pro Met Ser Trp Thr Cys Asp Lys Glu Asp Asp Cys Glu His
 2710 2715 2720
 ggc gag gac gag acc cac tgc aac aag ttc tgc tca gag gcc cag ttt
 8683
 Gly Glu Asp Glu Thr His Cys Asn Lys Phe Cys Ser Glu Ala Gln Phe
 2725 2730 2735
 gag tgc cag aac cat cgc tgc atc tcc aag cag tgg ctg tgt gac ggc
 8731
 Glu Cys Gln Asn His Arg Cys Ile Ser Lys Gln Trp Leu Cys Asp Gly
 2740 2745 2750 2755
 agc gat gac tgt ggg gat ggc tca gac gag gct gct cac tgt gaa ggc
 8779
 Ser Asp Asp Cys Gly Asp Gly Ser Asp Glu Ala Ala His Cys Glu Gly
 2760 2765 2770
 aag acg tgc ggc ccc tcc tcc ttc tcc tgc cct ggc acc cac gtg tgc
 8827
 Lys Thr Cys Gly Pro Ser Ser Phe Ser Cys Pro Gly Thr His Val Cys
 2775 2780 2785
 gtc ccc gag cgc tgg ctg tgt gac ggt gac aaa gac tgt gct gat ggt
 8875
 Val Pro Glu Arg Trp Leu Cys Asp Gly Asp Lys Asp Cys Ala Asp Gly
 2790 2795 2800
 gca gac gag agc atc gca gct ggt tgc ttg tac aac agc act tgt gac
 8923
 Ala Asp Glu Ser Ile Ala Ala Gly Cys Leu Tyr Asn Ser Thr Cys Asp
 2805 2810 2815
 gac cgt gag ttc atg tgc cag aac cgc cag tgc atc ccc aag cac ttc
 8971
 Asp Arg Glu Phe Met Cys Gln Asn Arg Gln Cys Ile Pro Lys His Phe
 2820 2825 2830 2835

- 38 -

gtg tgt gac cac gac cgt gac tgt gca gat ggc tct gat gag tcc ccc
9019

Val Cys Asp His Asp Arg Asp Cys Ala Asp Gly Ser Asp Glu Ser Pro
2840 2845 2850

gag tgt gag tac ccg acc tgc ggc ccc agt gag ttc cgc tgt gcc aat
9067

Glu Cys Glu Tyr Pro Thr Cys Gly Pro Ser Glu Phe Arg Cys Ala Asn
2855 2860 2865

ggg cgc tgt ctg agc tcc cgc cag tgg gag tgt gat ggc gag aat gac
9115

Gly Arg Cys Leu Ser Ser Arg Gln Trp Glu Cys Asp Gly Glu Asn Asp
2870 2875 2880

tgc cac gac cag agt gac gag gct ccc aag aac cca cac tgc acc agc
9163

Cys His Asp Gln Ser Asp Glu Ala Pro Lys Asn Pro His Cys Thr Ser
2885 2890 2895

cca gag cac aag tgc aat gcc tcg tca cag ttc ctg tgc agc agt ggg
9211

Pro Glu His Lys Cys Asn Ala Ser Ser Gln Phe Leu Cys Ser Ser Gly
2900 2905 2910 2915

cgc tgt gtg gct gag gca ctg ctc tgc aac ggc cag gat gac tgt ggc
9259

Arg Cys Val Ala Glu Ala Leu Leu Cys Asn Gly Gln Asp Asp Cys Gly
2920 2925 2930

gac agc tcg gac gag cgt ggc tgc cac atc aat gag tgt ctc agc cgc
9307

Asp Ser Ser Asp Glu Arg Gly Cys His Ile Asn Glu Cys Leu Ser Arg
2935 2940 2945

aag ctc agt ggc tgc agc cag gac tgt gag gac ctc aag atc ggc ttc
9355

Lys Leu Ser Gly Cys Ser Gln Asp Cys Glu Asp Leu Lys Ile Gly Phe
2950 2955 2960

aag tgc cgc tgt cgc cct ggc ttc cgg ctg aag gat gac ggc cgg acg
9403

Lys Cys Arg Cys Arg Pro Gly Phe Arg Leu Lys Asp Asp Gly Arg Thr
2965 2970 2975

tgt gct gat gtg gac gag tgc agc acc acc ttc ccc tgc agc cag cgc
9451

Cys Ala Asp Val Asp Glu Cys Ser Thr Thr Phe Pro Cys Ser Gln Arg
2980 2985 2990 2995

- 39 -

tgc atc aac acc cat ggc agc tat aag tgt ctg tgt gtg gag ggc tat
9499

Cys Ile Asn Thr His Gly Ser Tyr Lys Cys Leu Cys Val Glu Gly Tyr
3000 3005 3010

gca ccc cgc ggc ggc gac ccc cac agc tgc aag gct gtg act gac gag
9547

Ala Pro Arg Gly Gly Asp Pro His Ser Cys Lys Ala Val Thr Asp Glu
3015 3020 3025

gaa ccg ttt ctg atc ttc gcc aac cgg tac tac ctg cgc aag ctc aac
9595

Glu Pro Phe Leu Ile Phe Ala Asn Arg Tyr Tyr Leu Arg Lys Leu Asn
3030 3035 3040

ctg gac ggg tcc aac tac acg tta ctt aag cag ggc ctg aac aac gcc
9643

Leu Asp Gly Ser Asn Tyr Thr Leu Leu Lys Gln Gly Leu Asn Asn Ala
3045 3050 3055

gtt gcc ttg gat ttt gac tac cga gag cag atg atc tac tgg aca gat
9691

Val Ala Leu Asp Phe Asp Tyr Arg Glu Gln Met Ile Tyr Trp Thr Asp
3060 3065 3070 3075

gtg acc acc cag ggc agc atg atc cga agg atg cac ctt aac ggg agc
9739

Val Thr Thr Gln Gly Ser Met Ile Arg Arg Met His Leu Asn Gly Ser
3080 3085 3090

aat gtg cag gtc cta cac cgt aca ggc ctc agc aac ccc gat ggg ctg
9787

Asn Val Gln Val Leu His Arg Thr Gly Leu Ser Asn Pro Asp Gly Leu
3095 3100 3105

gct gtg gac tgg gtg ggt ggc aac ctg tac tgg tgc gac aaa ggc cgg
9835

Ala Val Asp Trp Val Gly Gly Asn Leu Tyr Trp Cys Asp Lys Gly Arg
3110 3115 3120

gac acc atc gag gtg tcc aag ctc aat ggg gcc tat cgg acg gtg ctg
9883

Asp Thr Ile Glu Val Ser Lys Leu Asn Gly Ala Tyr Arg Thr Val Leu
3125 3130 3135

gtc agc tct ggc ctc cgt gag ccc agg gct ctg gtg gtg gat gtg cag
9931

Val Ser Ser Gly Leu Arg Glu Pro Arg Ala Leu Val Val Asp Val Gln
3140 3145 3150 3155

aat ggg tac ctg tac tgg aca gac tgg ggt gac cat tca ctg atc ggc
9979

- 40 -

Asn Gly Tyr Leu Tyr Trp Thr Asp Trp Gly Asp His Ser Leu Ile Gly
 3160 3165 3170
 cgc atc ggc atg gat ggg tcc agc cgc agc gtc atc gtg gac acc aag
 10027
 Arg Ile Gly Met Asp Gly Ser Ser Arg Ser Val Ile Val Asp Thr Lys
 3175 3180 3185
 atc aca tgg ccc aat ggc ctg acg ctg gac tat gtc act gag cgc atc
 10075
 Ile Thr Trp Pro Asn Gly Leu Thr Leu Asp Tyr Val Thr Glu Arg Ile
 3190 3195 3200
 tac tgg gcc gac gcc cgc gag gac tac att gaa ttt gcc agc ctg gat
 10123
 Tyr Trp Ala Asp Ala Arg Glu Asp Tyr Ile Glu Phe Ala Ser Leu Asp
 3205 3210 3215
 ggc tcc aat cgc cac gtt gtg ctg agc cag gac atc ccg cac atc ttt
 10171
 Gly Ser Asn Arg His Val Val Leu Ser Gln Asp Ile Pro His Ile Phe
 3220 3225 3230 3235
 gca ctg acc ctg ttt gag gac tac gtc tac tgg acc gac tgg gaa aca
 10219
 Ala Leu Thr Leu Phe Glu Asp Tyr Val Tyr Trp Thr Asp Trp Glu Thr
 3240 3245 3250
 aag tcc att aac cga gcc cac aag acc acg ggc acc aac aaa acg ctc
 10267
 Lys Ser Ile Asn Arg Ala His Lys Thr Thr Gly Thr Asn Lys Thr Leu
 3255 3260 3265
 ctc atc agc acg ctg cac cgg ccc atg gac ctg cat gtc ttc cat gcc
 10315
 Leu Ile Ser Thr Leu His Arg Pro Met Asp Leu His Val Phe His Ala
 3270 3275 3280
 ctg cgc cag cca gac gtg ccc aat cac ccc tgc aag gtc aac aat ggt
 10363
 Leu Arg Gln Pro Asp Val Pro Asn His Pro Cys Lys Val Asn Asn Gly
 3285 3290 3295
 ggc tgc agc aac ctg tgc ctg ctg tcc ccc ggg gga ggg cac aaa tgt
 10411
 Gly Cys Ser Asn Leu Cys Leu Leu Ser Pro Gly Gly Gly His Lys Cys
 3300 3305 3310 3315
 gcc tgc ccc acc aac ttc tac ctg ggc agc gat ggg cgc acc tgt gtg
 10459
 Ala Cys Pro Thr Asn Phe Tyr Leu Gly Ser Asp Gly Arg Thr Cys Val
 3320 3325 3330

- 41 -

tcc aac tgc acg gct agc cag ttt gta tgc aag aac gac aag tgc atc
10507

Ser Asn Cys Thr Ala Ser Gln Phe Val Cys Lys Asn Asp Lys Cys Ile
3335 3340 3345

ccc ttc tgg tgg aag tgt gac acc gag gac gac tgc ggg gac cac tca
10555

Pro Phe Trp Trp Lys Cys Asp Thr Glu Asp Asp Cys Gly Asp His Ser
3350 3355 3360

gac gag ccc ccg gac tgc cct gag ttc aag tgc cgg ccc gga cag ttc
10603

Asp Glu Pro Pro Asp Cys Pro Glu Phe Lys Cys Arg Pro Gly Gln Phe
3365 3370 3375

cag tgc tcc aca ggt atc tgc aca aac cct gcc ttc atc tgc gat ggc
10651

Gln Cys Ser Thr Gly Ile Cys Thr Asn Pro Ala Phe Ile Cys Asp Gly
3380 3385 3390 3395

gac aat gac tgc cag gac aac agt gac gag gcc aac tgt gac atc cac
10699

Asp Asn Asp Cys Gln Asp Asn Ser Asp Glu Ala Asn Cys Asp Ile His
3400 3405 3410

gtc tgc ttg ccc agt cag ttc aaa tgc acc aac acc aac cgc tgt att
10747

Val Cys Leu Pro Ser Gln Phe Lys Cys Thr Asn Thr Asn Arg Cys Ile
3415 3420 3425

ccc ggc atc ttc cgc tgc aat ggg cag gac aac tgc gga gat ggg gag
10795

Pro Gly Ile Phe Arg Cys Asn Gly Gln Asp Asn Cys Gly Asp Gly Glu
3430 3435 3440

gat gag agg gac tgc ccc gag gtg acc tgc gcc ccc aac cag ttc cag
10843

Asp Glu Arg Asp Cys Pro Glu Val Thr Cys Ala Pro Asn Gln Phe Gln
3445 3450 3455

tgc tcc att acc aaa cgg tgc atc ccc cgg gtc tgg gtc tgc gac cgg
10891

Cys Ser Ile Thr Lys Arg Cys Ile Pro Arg Val Trp Val Cys Asp Arg
3460 3465 3470 3475

gac aat gac tgt gtg gat ggc agt gat gag ccc gcc aac tgc acc cag
10939

Asp Asn Asp Cys Val Asp Gly Ser Asp Glu Pro Ala Asn Cys Thr Gln
3480 3485 3490

- 42 -

atg acc tgt ggt gtg gac gag ttc cgc tgc aag gat tcg ggc cgc tgc
10987

Met Thr Cys Gly Val Asp Glu Phe Arg Cys Lys Asp Ser Gly Arg Cys
3495 3500 3505

atc cca gcg cgt tgg aag tgt gac gga gag gat gac tgt ggg gat ggc
11035

Ile Pro Ala Arg Trp Lys Cys Asp Gly Glu Asp Asp Cys Gly Asp Gly
3510 3515 3520

tcg gat gag ccc aag gaa gag tgt gat gaa cgc acc tgt gag cca tac
11083

Ser Asp Glu Pro Lys Glu Glu Cys Asp Glu Arg Thr Cys Glu Pro Tyr
3525 3530 3535

cag ttc cgc tgc aag aac aac cgc tgc gtg ccc ggc cgc tgg cag tgc
11131

Gln Phe Arg Cys Lys Asn Asn Arg Cys Val Pro Gly Arg Trp Gln Cys
3540 3545 3550 3555

gac tac gac aac gat tgc ggt gac aac tcc gat gaa gag agc tgc acc
11179

Asp Tyr Asp Asn Asp Cys Gly Asp Asn Ser Asp Glu Glu Ser Cys Thr
3560 3565 3570

cct cgg ccc tgc tcc gag agt gag ttc tcc tgt gcc aac ggc cgc tgc
11227

Pro Arg Pro Cys Ser Glu Ser Glu Phe Ser Cys Ala Asn Gly Arg Cys
3575 3580 3585

atc gcg ggg cgc tgg aaa tgc gat gga gac cac gac tgc gcg gac ggc
11275

Ile Ala Gly Arg Trp Lys Cys Asp Gly Asp His Asp Cys Ala Asp Gly
3590 3595 3600

tcg gac gag aaa gac tgc acc ccc cgc tgt gac atg gac cag ttc cag
11323

Ser Asp Glu Lys Asp Cys Thr Pro Arg Cys Asp Met Asp Gln Phe Gln
3605 3610 3615

tgc aag agc ggc cac tgc atc ccc ctg cgc tgg cgc tgt gac gca gac
11371

Cys Lys Ser Gly His Cys Ile Pro Leu Arg Trp Arg Cys Asp Ala Asp
3620 3625 3630 3635

gcc gac tgc atg gac ggc agc gac gag gag gcc tgc ggc act ggc gtg
11419

Ala Asp Cys Met Asp Gly Ser Asp Glu Glu Ala Cys Gly Thr Gly Val
3640 3645 3650

cgg acc tgc ccc ctg gac gag ttc cag tgc aac aac acc ttg tgc aag
11467

- 43 -

Arg Thr Cys Pro Leu Asp Glu Phe Gln Cys Asn Asn Thr Leu Cys Lys
 3655 3660 3665

cgc ctg gcc tgg aag tgc gat ggc gag gat gac tgt ggg gac aac tca
 11515

Pro Leu Ala Trp Lys Cys Asp Gly Glu Asp Asp Cys Gly Asp Asn Ser
 3670 3675 3680

gat gag aac ccc gag gag tgt gcc cgg ttc gtg tgc cct ccc aac cgg
 11563

Asp Glu Asn Pro Glu Glu Cys Ala Arg Phe Val Cys Pro Pro Asn Arg
 3685 3690 3695

ccc ttc cgt tgc aag aat gac cgc gtc tgt ctg tgg atc ggg cgc caa
 11611

Pro Phe Arg Cys Lys Asn Asp Arg Val Cys Leu Trp Ile Gly Arg Gln
 3700 3705 3710 3715

tgc gat ggc acg gac aac tgt ggg gat ggg act gat gaa gag gac tgt
 11659

Cys Asp Gly Thr Asp Asn Cys Gly Asp Gly Thr Asp Glu Glu Asp Cys
 3720 3725 3730

gag ccc ccc aca gcc cac acc acc cac tgc aaa gac aag aag gag ttt
 11707

Glu Pro Pro Thr Ala His Thr Thr His Cys Lys Asp Lys Lys Glu Phe
 3735 3740 3745

ctg tgc cgg aac cag cgc tgc ctc tcc tcc tcc ctg cgc tgc aac atg
 11755

Leu Cys Arg Asn Gln Arg Cys Leu Ser Ser Ser Leu Arg Cys Asn Met
 3750 3755 3760

ttc gat gac tgc ggg gac ggc tct gac gag gag gac tgc agc atc gac
 11803

Phe Asp Asp Cys Gly Asp Gly Ser Asp Glu Glu Asp Cys Ser Ile Asp
 3765 3770 3775

ccc aag ctg acc agc tgc gcc acc aat gcc agc atc tgt ggg gac gag
 11851

Pro Lys Leu Thr Ser Cys Ala Thr Asn Ala Ser Ile Cys Gly Asp Glu
 3780 3785 3790 3795

gca cgc tgc gtg cgc acc gag aaa gcg gcc tac tgt gcc tgc cgc tgc
 11899

Ala Arg Cys Val Arg Thr Glu Lys Ala Ala Tyr Cys Ala Cys Arg Ser
 3800 3805 3810

ggc ttc cac acc gtg ccc ggc cag ccc gga tgc caa gac atc aac gag
 11947

Gly Phe His Thr Val Pro Gly Gln Pro Gly Cys Gln Asp Ile Asn Glu
 3815 3820 3825

- 44 -

tgc ctg cgc ttc ggc acc tgc tcc cag ctc tgc aac aac acc aag ggc
11995

Cys Leu Arg Phe Gly Thr Cys Ser Gln Leu Cys Asn Asn Thr Lys Gly
3830 3835 3840

ggc cac ctc tgc agc tgc gct cgg aac ttc atg aag acg cac aac acc
12043

Gly His Leu Cys Ser Cys Ala Arg Asn Phe Met Lys Thr His Asn Thr
3845 3850 3855

tgc aag gcc gaa ggc tct gag tac cag gtc ctg tac atc gct gat gac
12091

Cys Lys Ala Glu Gly Ser Glu Tyr Gln Val Leu Tyr Ile Ala Asp Asp
3860 3865 3870 3875

aat gag atc cgc agc ctg ttc ccc ggc cac ccc cat tcg gct tac gag
12139

Asn Glu Ile Arg Ser Leu Phe Pro Gly His Pro His Ser Ala Tyr Glu
3880 3885 3890

cag gca ttc cag ggt gac gag agt gtc cgc att gat gct atg gat gtc
12187

Gln Ala Phe Gln Gly Asp Glu Ser Val Arg Ile Asp Ala Met Asp Val
3895 3900 3905

cat gtc aag gct ggc cgt gtc tat tgg acc aac tgg cac acg ggc acc
12235

His Val Lys Ala Gly Arg Val Tyr Trp Thr Asn Trp His Thr Gly Thr
3910 3915 3920

atc tcc tac cgc agc ctg cca cct gct gcg cct cct acc act tcc aac
12283

Ile Ser Tyr Arg Ser Leu Pro Pro Ala Ala Pro Pro Thr Thr Ser Asn
3925 3930 3935

cgc cac cgg cga cag att gac cgg ggt gtc acc cac ctc aac att tca
12331

Arg His Arg Arg Gln Ile Asp Arg Gly Val Thr His Leu Asn Ile Ser
3940 3945 3950 3955

ggg ctg aag atg ccc aga ggc atc gcc atc gac tgg gtg gcc gga aac
12379

Gly Leu Lys Met Pro Arg Gly Ile Ala Ile Asp Trp Val Ala Gly Asn
3960 3965 3970

gtg tac tgg acc gac tcg ggc cga gat gtg att gag gtg gcg cag atg
12427

Val Tyr Trp Thr Asp Ser Gly Arg Asp Val Ile Glu Val Ala Gln Met
3975 3980 3985

- 45 -

aag ggc gag aac cgc aag acg ctc atc tcg ggc atg att gac gag ccc
12475
Lys Gly Glu Asn Arg Lys Thr Leu Ile Ser Gly Met Ile Asp Glu Pro
3990 3995 4000

cac gcc att gtg gtg gac cca ctg agg ggg acc atg tac tgg tca gac
12523
His Ala Ile Val Val Asp Pro Leu Arg Gly Thr Met Tyr Trp Ser Asp
4005 4010 4015

tgg ggc aac cac ccc aag att gag acg gca gcg atg gat ggg acg ctt
12571
Trp Gly Asn His Pro Lys Ile Glu Thr Ala Ala Met Asp Gly Thr Leu
4020 4025 4030 4035

cgg gag aca ctg gtg cag gac aac att cag tgg ccc aca ggc ctg gcc
12619
Arg Glu Thr Leu Val Gln Asp Asn Ile Gln Trp Pro Thr Gly Leu Ala
4040 4045 4050

gtg gat tat cac aat gag cgg ctg tac tgg gca gac gcc aag ctt tca
12667
Val Asp Tyr His Asn Glu Arg Leu Tyr Trp Ala Asp Ala Lys Leu Ser
4055 4060 4065

gtc atc ggc agc atc cgg ctc aat ggc acg gac ccc att gtg gct gct
12715
Val Ile Gly Ser Ile Arg Leu Asn Gly Thr Asp Pro Ile Val Ala Ala
4070 4075 4080

gac agc aaa cga ggc cta agt cac ccc ttc agc atc gac gtc ttt gag
12763
Asp Ser Lys Arg Gly Leu Ser His Pro Phe Ser Ile Asp Val Phe Glu
4085 4090 4095

gat tac atc tat ggt gtc acc tac atc aat aat cgt gtc ttc aag atc
12811
Asp Tyr Ile Tyr Gly Val Thr Tyr Ile Asn Asn Arg Val Phe Lys Ile
4100 4105 4110 4115

cat aag ttt ggc cac agc ccc ttg gtc aac ctg aca ggg ggc ctg agc
12859
His Lys Phe Gly His Ser Pro Leu Val Asn Leu Thr Gly Gly Leu Ser
4120 4125 4130

cac gcc tct gac gtg gtc ctt tac cat cag cac aag cag ccc gaa gtg
12907
His Ala Ser Asp Val Val Leu Tyr His Gln His Lys Gln Pro Glu Val
4135 4140 4145

acc aac cca tgt gac cgc aag aaa tgc gag tgg ctc tgc ctg ctg agc
12955

- 46 -

Thr Asn Pro Cys Asp Arg Lys Lys Cys Glu Trp Leu Cys Leu Leu Ser
 4150 4155 4160
 ccc agt ggg cct gtc tgc acc tgt ccc aat ggg aag cgg ctg gac aac
 13003
 Pro Ser Gly Pro Val Cys Thr Cys Pro Asn Gly Lys Arg Leu Asp Asn
 4165 4170 4175
 ggc aca tgc gtg cct gtg ccc tct cca acg ccc ccc cca gat gct ccc
 13051
 Gly Thr Cys Val Pro Val Pro Ser Pro Thr Pro Pro Pro Asp Ala Pro
 4180 4185 4190 4195
 cgg cct gga acc tgt aac ctg cag tgc ttc aac ggt ggc agc tgt ttc
 13099
 Arg Pro Gly Thr Cys Asn Leu Gln Cys Phe Asn Gly Gly Ser Cys Phe
 4200 4205 4210
 ctc aat gca cgg agg cag ccc aag tgc cgc tgc caa ccc cgc tac acg
 13147
 Leu Asn Ala Arg Arg Gln Pro Lys Cys Arg Cys Gln Pro Arg Tyr Thr
 4215 4220 4225
 ggt gac aag tgt gaa ctg gac cag tgc tgg gag cac tgt cgc aat ggg
 13195
 Gly Asp Lys Cys Glu Leu Asp Gln Cys Trp Glu His Cys Arg Asn Gly
 4230 4235 4240
 ggc acc tgt gct gcc tcc ccc tct ggc atg ccc acg tgc cgg tgc ccc
 13243
 Gly Thr Cys Ala Ala Ser Pro Ser Gly Met Pro Thr Cys Arg Cys Pro
 4245 4250 4255
 acg ggc ttc acg ggc ccc aaa tgc acc cag cag gtg tgt gcg ggc tac
 13291
 Thr Gly Phe Thr Gly Pro Lys Cys Thr Gln Gln Val Cys Ala Gly Tyr
 4260 4265 4270 4275
 tgt gcc aac aac agc acc tgc act gtc aac cag ggc aac cag ccc cag
 13339
 Cys Ala Asn Asn Ser Thr Cys Thr Val Asn Gln Gly Asn Gln Pro Gln
 4280 4285 4290
 tgc cga tgc cta ccc ggc ttc ctg ggc gac cgc tgc cag tac cgg cag
 13387
 Cys Arg Cys Leu Pro Gly Phe Leu Gly Asp Arg Cys Gln Tyr Arg Gln
 4295 4300 4305
 tgc tct ggc tac tgt gag aac ttt ggc aca tgc cag atg gct gct gat
 13435
 Cys Ser Gly Tyr Cys Glu Asn Phe Gly Thr Cys Gln Met Ala Ala Asp
 4310 4315 4320

- 47 -

ggc tcc cga caa tgc cgc tgc act gcc tac ttt gag gga tcg agg tgt
 13483
 Gly Ser Arg Gln Cys Arg Cys Thr Ala Tyr Phe Glu Gly Ser Arg Cys
 4325 4330 4335

gag gtg aac aag tgc agc cgc tgt ctc gaa ggg gcc tgt gtg gtc aac
 13531
 Glu Val Asn Lys Cys Ser Arg Cys Leu Glu Gly Ala Cys Val Val Asn
 4340 4345 4350 4355

aag cag agt ggg gat gtc acc tgc aac tgc acg gat ggc cgg gtg gcc
 13579
 Lys Gln Ser Gly Asp Val Thr Cys Asn Cys Thr Asp Gly Arg Val Ala
 4360 4365 4370

ccc agc tgt ctg acc tgc gtc ggc cac tgc agc aat ggc ggc tcc tgt
 13627
 Pro Ser Cys Leu Thr Cys Val Gly His Cys Ser Asn Gly Gly Ser Cys
 4375 4380 4385

acc atg aac agc aaa atg atg cct gag tgc cag tgc cca ccc cac atg
 13675
 Thr Met Asn Ser Lys Met Met Pro Glu Cys Gln Cys Pro Pro His Met
 4390 4395 4400

aca ggg ccc cgg tgt gag gag cac gtc ttc agc cag cag cag cca gga
 13723
 Thr Gly Pro Arg Cys Glu Glu His Val Phe Ser Gln Gln Gln Pro Gly
 4405 4410 4415

cat ata gcc tcc atc cta atc cct ctg ctg ttg ctg ctg ctg gtt
 13771
 His Ile Ala Ser Ile Leu Ile Pro Leu Leu Leu Leu Leu Leu Val
 4420 4425 4430 4435

ctg gtg gcc gga gtg gta ttc tgg tat aag cgg cga gtc caa ggg gct
 13819
 Leu Val Ala Gly Val Val Phe Trp Tyr Lys Arg Arg Val Gln Gly Ala
 4440 4445 4450

aag ggc ttc cag cac caa cgg atg acc aac ggg gcc atg aac gtg gag
 13867
 Lys Gly Phe Gln His Gln Arg Met Thr Asn Gly Ala Met Asn Val Glu
 4455 4460 4465

att gga aac ccc acc tac aag atg tac gaa ggc gga gag cct gat gat
 13915
 Ile Gly Asn Pro Thr Tyr Lys Met Tyr Glu Gly Gly Glu Pro Asp Asp
 4470 4475 4480

- 48 -

gtg gga ggc cta ctg gac gct gac ttt gcc ctg gac cct gac aag ccc
13963

Val Gly Gly Leu Leu Asp Ala Asp Phe Ala Leu Asp Pro Asp Lys Pro
4485 4490 4495

acc aac ttc acc aac ccc gtg tat gcc aca ctc tac atg ggg ggc cat
14011

Thr Asn Phe Thr Asn Pro Val Tyr Ala Thr Leu Tyr Met Gly Gly His
4500 4505 4510 4515

ggc agt cgc cac tcc ctg gcc agc acg gac gag aag cga gaa ctc ctg
14059

Gly Ser Arg His Ser Leu Ala Ser Thr Asp Glu Lys Arg Glu Leu Leu
4520 4525 4530

ggc cgg ggc cct gag gac gag ata ggg gac ccc ttg gca tag
14101

Gly Arg Gly Pro Glu Asp Glu Ile Gly Asp Pro Leu Ala
4535 4540

ggccctgccc cgtcggactg cccccagaaa gcctcctgcc cctgcgggt gaagtccttc
14161

agtgcgcccc tccccagcca gcccttcctt gggcccgccg gatgtataaa tgtaaaaatg
14221

aagggaattac attttatatg tgagcgagca agccggcaag cgagcacagt attatttctc
14281

catccctcc ctgcctgctc cttggcacc ccattgctgcc ttcagggaga caggcagggg
14341

gggcttgggg ctgcacctcc taccctccca ccagaacgca cccactggg agagctggtg
14401

gtgcagcctt cccctccctg tataagacac tttgccaagg ctctccctc tcgccccatc
14461

cctgcttgcc cgtcccaaca gcttctgag ggctaattct ggaaggag agttctttgc
14521

tgccctgtc tggaagacgt ggctctgggt gaggtaggcg ggaaaggatg gagtgtttta
14581

gttcttgggg gaggccacc caaacccag ccccaactcc aggggcacct atgagatggc
14641

catgctcaac cccctccca gacaggccct cctgtctcc agggcccca ccgaggttcc
14701

cagggtgga gacttcctct ggtaaacatt cctccagcct cccctccctt ggggacgcca
14761

aggaggtggg ccacaccag gaagggaag cgggcagccc cgttttggg acgtgaacgt
14821

tttaataatt tttgctgaat tctttacaac taaataacac agatattctt ataaataaaa
14881

ttgtaaaaaa aaaaa
14896

<210> 10

<211> 4544

<212> PRT

- 49 -

<213> Homo sapiens

<400> 10

```

Met Leu Thr Pro Pro Leu Leu Leu Leu Leu Pro Leu Leu Ser Ala Leu
 1           5           10           15
Val Ala Ala Ala Ile Asp Ala Pro Lys Thr Cys Ser Pro Lys Gln Phe
      20           25           30
Ala Cys Arg Asp Gln Ile Thr Cys Ile Ser Lys Gly Trp Arg Cys Asp
      35           40           45
Gly Glu Arg Asp Cys Pro Asp Gly Ser Asp Glu Ala Pro Glu Ile Cys
      50           55           60
Pro Gln Ser Lys Ala Gln Arg Cys Gln Pro Asn Glu His Asn Cys Leu
      65           70           75           80
Gly Thr Glu Leu Cys Val Pro Met Ser Arg Leu Cys Asn Gly Val Gln
      85           90           95
Asp Cys Met Asp Gly Ser Asp Glu Gly Pro His Cys Arg Glu Leu Gln
      100          105          110
Gly Asn Cys Ser Arg Leu Gly Cys Gln His His Cys Val Pro Thr Leu
      115          120          125
Asp Gly Pro Thr Cys Tyr Cys Asn Ser Ser Phe Gln Leu Gln Ala Asp
      130          135          140
Gly Lys Thr Cys Lys Asp Phe Asp Glu Cys Ser Val Tyr Gly Thr Cys
      145          150          155          160
Ser Gln Leu Cys Thr Asn Thr Asp Gly Ser Phe Ile Cys Gly Cys Val
      165          170          175
Glu Gly Tyr Leu Leu Gln Pro Asp Asn Arg Ser Cys Lys Ala Lys Asn
      180          185          190
Glu Pro Val Asp Arg Pro Pro Val Leu Leu Ile Ala Asn Ser Gln Asn
      195          200          205
Ile Leu Ala Thr Tyr Leu Ser Gly Ala Gln Val Ser Thr Ile Thr Pro
      210          215          220
Thr Ser Thr Arg Gln Thr Thr Ala Met Asp Phe Ser Tyr Ala Asn Glu
      225          230          235          240
Thr Val Cys Trp Val His Val Gly Asp Ser Ala Ala Gln Thr Gln Leu
      245          250          255
Lys Cys Ala Arg Met Pro Gly Leu Lys Gly Phe Val Asp Glu His Thr
      260          265          270
Ile Asn Ile Ser Leu Ser Leu His His Val Glu Gln Met Ala Ile Asp
      275          280          285
Trp Leu Thr Gly Asn Phe Tyr Phe Val Asp Asp Ile Asp Asp Arg Ile
      290          295          300
Phe Val Cys Asn Arg Asn Gly Asp Thr Cys Val Thr Leu Leu Asp Leu
      305          310          315          320
Glu Leu Tyr Asn Pro Lys Gly Ile Ala Leu Asp Pro Ala Met Gly Lys
      325          330          335
Val Phe Phe Thr Asp Tyr Gly Gln Ile Pro Lys Val Glu Arg Cys Asp
      340          345          350
Met Asp Gly Gln Asn Arg Thr Lys Leu Val Asp Ser Lys Ile Val Phe
      355          360          365
Pro His Gly Ile Thr Leu Asp Leu Val Ser Arg Leu Val Tyr Trp Ala
      370          375          380
Asp Ala Tyr Leu Asp Tyr Ile Glu Val Val Asp Tyr Glu Gly Lys Gly

```

- 50 -

```

385              390              395              400
Arg Gln Thr Ile Ile Gln Gly Ile Leu Ile Glu His Leu Tyr Gly Leu
              405              410              415
Thr Val Phe Glu Asn Tyr Leu Tyr Ala Thr Asn Ser Asp Asn Ala Asn
              420              425              430
Ala Gln Gln Lys Thr Ser Val Ile Arg Val Asn Arg Phe Asn Ser Thr
              435              440              445
Glu Tyr Gln Val Val Thr Arg Val Asp Lys Gly Gly Ala Leu His Ile
              450              455              460
Tyr His Gln Arg Arg Gln Pro Arg Val Arg Ser His Ala Cys Glu Asn
465              470              475              480
Asp Gln Tyr Gly Lys Pro Gly Gly Cys Ser Asp Ile Cys Leu Leu Ala
              485              490              495
Asn Ser His Lys Ala Arg Thr Cys Arg Cys Arg Ser Gly Phe Ser Leu
              500              505              510
Gly Ser Asp Gly Lys Ser Cys Lys Lys Pro Glu His Glu Leu Phe Leu
              515              520              525
Val Tyr Gln Lys Gly Arg Pro Gly Ile Ile Arg Gly Met Asp Met Gly
              530              535              540
Ala Lys Val Pro Asp Glu His Met Ile Pro Ile Glu Asn Leu Met Asn
545              550              555              560
Pro Arg Ala Leu Asp Phe His Ala Glu Thr Gly Phe Ile Tyr Phe Ala
              565              570              575
Asp Thr Thr Ser Tyr Leu Ile Gly Arg Gln Lys Ile Asp Gly Thr Glu
              580              585              590
Arg Glu Thr Ile Leu Lys Asp Gly Ile His Asn Val Glu Gly Val Ala
              595              600              605
Val Asp Trp Met Gly Asp Asn Leu Tyr Trp Thr Asp Asp Gly Pro Lys
              610              615              620
Lys Thr Ile Ser Val Ala Arg Leu Glu Lys Ala Ala Gln Thr Arg Lys
625              630              635              640
Thr Leu Ile Glu Gly Lys Met Thr His Pro Arg Ala Ile Val Val Asp
              645              650              655
Pro Leu Asn Gly Trp Met Tyr Trp Thr Asp Trp Glu Glu Asp Pro Lys
              660              665              670
Asp Ser Arg Arg Gly Arg Leu Glu Arg Ala Trp Met Asp Gly Ser His
              675              680              685
Arg Asp Ile Phe Val Thr Ser Lys Thr Val Leu Trp Pro Asn Gly Leu
              690              695              700
Ser Leu Asp Ile Pro Ala Gly Arg Leu Tyr Trp Val Asp Ala Phe Tyr
705              710              715              720
Asp Arg Ile Glu Thr Ile Leu Leu Asn Gly Thr Asp Arg Lys Ile Val
              725              730              735
Tyr Glu Gly Pro Glu Leu Asn His Ala Phe Gly Leu Cys His His Gly
              740              745              750
Asn Tyr Leu Phe Trp Thr Glu Tyr Arg Ser Gly Ser Val Tyr Arg Leu
              755              760              765
Glu Arg Gly Val Gly Gly Ala Pro Pro Thr Val Thr Leu Leu Arg Ser
              770              775              780
Glu Arg Pro Pro Ile Phe Glu Ile Arg Met Tyr Asp Ala Gln Gln Gln
785              790              795              800
Gln Val Gly Thr Asn Lys Cys Arg Val Asn Asn Gly Gly Cys Ser Ser

```


805										810				815			
Leu	Cys	Leu	Ala	Thr	Pro	Gly	Ser	Arg	Gln	Cys	Ala	Cys	Ala	Glu	Asp		
820				840				860				880					
Gln	Val	Leu	Asp	Ala	Asp	Gly	Val	Thr	Cys	Leu	Ala	Asn	Pro	Ser	Tyr		
835				855				875				895					
Val	Pro	Pro	Pro	Gln	Cys	Gln	Pro	Gly	Glu	Phe	Ala	Cys	Ala	Asn	Ser		
850				870				890				910					
Arg	Cys	Ile	Gln	Glu	Arg	Trp	Lys	Cys	Asp	Gly	Asp	Asn	Asp	Cys	Leu		
865				885				905				925					
Asp	Asn	Ser	Asp	Glu	Ala	Pro	Ala	Leu	Cys	His	Gln	His	Thr	Cys	Pro		
880				900				920				940					
Ser	Asp	Arg	Phe	Lys	Cys	Glu	Asn	Asn	Arg	Cys	Ile	Pro	Asn	Arg	Trp		
900				920				940				960					
Leu	Cys	Asp	Gly	Asp	Asn	Asp	Cys	Gly	Asn	Ser	Glu	Asp	Glu	Ser	Asn		
915				935				955				975					
Ala	Thr	Cys	Ser	Ala	Arg	Thr	Cys	Pro	Pro	Asn	Gln	Phe	Ser	Cys	Ala		
930				950				970				990					
Ser	Gly	Arg	Cys	Ile	Pro	Ile	Ser	Trp	Thr	Cys	Asp	Leu	Asp	Asp	Asp		
945				965				985				1005					
Cys	Gly	Asp	Arg	Ser	Asp	Glu	Ser	Ala	Ser	Cys	Ala	Tyr	Pro	Thr	Cys		
960				980				1000				1020					
Phe	Pro	Leu	Thr	Gln	Phe	Thr	Cys	Asn	Asn	Gly	Arg	Cys	Ile	Asn	Ile		
980				1000				1020				1040					
Asn	Trp	Arg	Cys	Asp	Asn	Asp	Asn	Asp	Cys	Gly	Asp	Asn	Ser	Asp	Glu		
995				1015				1035				1055					
Ala	Gly	Cys	Ser	His	Ser	Cys	Ser	Ser	Thr	Gln	Phe	Lys	Cys	Asn	Ser		
1010				1030				1050				1070					
Gly	Arg	Cys	Ile	Pro	Glu	His	Trp	Thr	Cys	Asp	Gly	Asp	Asn	Asp	Cys		
1025				1045				1065				1085					
Gly	Asp	Tyr	Ser	Asp	Glu	Thr	His	Ala	Asn	Cys	Thr	Asn	Gln	Ala	Thr		
1040				1060				1080				1100					
Arg	Pro	Pro	Gly	Gly	Cys	His	Thr	Asp	Glu	Phe	Gln	Cys	Arg	Leu	Asp		
1060				1080				1100				1120					
Gly	Leu	Cys	Ile	Pro	Leu	Arg	Trp	Arg	Cys	Asp	Gly	Asp	Thr	Asp	Cys		
1075				1095				1115				1135					
Met	Asp	Ser	Ser	Asp	Glu	Lys	Ser	Cys	Glu	Gly	Val	Thr	His	Val	Cys		
1090				1110				1130				1150					
Asp	Pro	Ser	Val	Lys	Phe	Gly	Cys	Lys	Asp	Ser	Ala	Arg	Cys	Ile	Ser		
1105				1125				1145				1165					
Lys	Ala	Trp	Val	Cys	Asp	Gly	Asp	Asn	Asp	Cys	Glu	Asp	Asn	Ser	Asp		
1120				1140				1160				1180					
Glu	Glu	Asn	Cys	Glu	Ser	Leu	Ala	Cys	Arg	Pro	Pro	Ser	His	Pro	Cys		
1140				1160				1180				1200					
Ala	Asn	Asn	Thr	Ser	Val	Cys	Leu	Pro	Pro	Asp	Lys	Leu	Cys	Asp	Gly		
1155				1175				1195				1215					
Asn	Asp	Asp	Cys	Gly	Asp	Gly	Ser	Asp	Glu	Gly	Glu	Leu	Cys	Asp	Gln		
1170				1190				1210				1230					
Cys	Ser	Leu	Asn	Asn	Gly	Gly	Cys	Ser	His	Asn	Cys	Ser	Val	Ala	Pro		
1185				1205				1225				1245					
Gly	Glu	Gly	Ile	Val	Cys	Ser	Cys	Pro	Leu	Gly	Met	Glu	Leu	Gly	Pro		
1205				1225				1245				1265					
Asp	Asn	His	Thr	Cys	Gln	Ile	Gln	Ser	Tyr	C							

1220										1225					1230				
Cys	Ser	Gln	Lys	Cys	Asp	Gln	Asn	Lys	Phe	Ser	Val	Lys	Cys	Ser	Cys				
1235										1240					1245				
Tyr	Glu	Gly	Trp	Val	Leu	Glu	Pro	Asp	Gly	Glu	Ser	Cys	Arg	Ser	Leu				
1250										1255					1260				
Asp	Pro	Phe	Lys	Pro	Phe	Ile	Ile	Phe	Ser	Asn	Arg	His	Glu	Ile	Arg				
1265										1270					1275				
Arg	Ile	Asp	Leu	His	Lys	Gly	Asp	Tyr	Ser	Val	Leu	Val	Pro	Gly	Leu				
1285										1290					1295				
Arg	Asn	Thr	Ile	Ala	Leu	Asp	Phe	His	Leu	Ser	Gln	Ser	Ala	Leu	Tyr				
1300										1305					1310				
Trp	Thr	Asp	Val	Val	Glu	Asp	Lys	Ile	Tyr	Arg	Gly	Lys	Leu	Leu	Asp				
1315										1320					1325				
Asn	Gly	Ala	Leu	Thr	Ser	Phe	Glu	Val	Val	Ile	Gln	Tyr	Gly	Leu	Ala				
1330										1335					1340				
Thr	Pro	Glu	Gly	Leu	Ala	Val	Asp	Trp	Ile	Ala	Gly	Asn	Ile	Tyr	Trp				
1345										1350					1355				
Val	Glu	Ser	Asn	Leu	Asp	Gln	Ile	Glu	Val	Ala	Lys	Leu	Asp	Gly	Thr				
1365										1370					1375				
Leu	Arg	Thr	Thr	Leu	Leu	Ala	Gly	Asp	Ile	Glu	His	Pro	Arg	Ala	Ile				
1380										1385					1390				
Ala	Leu	Asp	Pro	Arg	Asp	Gly	Ile	Leu	Phe	Trp	Thr	Asp	Trp	Asp	Ala				
1395										1400					1405				
Ser	Leu	Pro	Arg	Ile	Glu	Ala	Ala	Ser	Met	Ser	Gly	Ala	Gly	Arg	Arg				
1410										1415					1420				
Thr	Val	His	Arg	Glu	Thr	Gly	Ser	Gly	Gly	Trp	Pro	Asn	Gly	Leu	Thr				
1425										1430					1435				
Val	Asp	Tyr	Leu	Glu	Lys	Arg	Ile	Leu	Trp	Ile	Asp	Ala	Arg	Ser	Asp				
1445										1450					1455				
Ala	Ile	Tyr	Ser	Ala	Arg	Tyr	Asp	Gly	Ser	Gly	His	Met	Glu	Val	Leu				
1460										1465					1470				
Arg	Gly	His	Glu	Phe	Leu	Ser	His	Pro	Phe	Ala	Val	Thr	Leu	Tyr	Gly				
1475										1480					1485				
Gly	Glu	Val	Tyr	Trp	Thr	Asp	Trp	Arg	Thr	Asn	Thr	Leu	Ala	Lys	Ala				
1490										1495					1500				
Asn	Lys	Trp	Thr	Gly	His	Asn	Val	Thr	Val	Val	Gln	Arg	Thr	Asn	Thr				
1505										1510					1515				
Gln	Pro	Phe	Asp	Leu	Gln	Val	Tyr	His	Pro	Ser	Arg	Gln	Pro	Met	Ala				
1525										1530					1535				
Pro	Asn	Pro	Cys	Glu	Ala	Asn	Gly	Gly	Gln	Gly	Pro	Cys	Ser	His	Leu				
1540										1545					1550				
Cys	Leu	Ile	Asn	Tyr	Asn	Arg	Thr	Val	Ser	Cys	Ala	Cys	Pro	His	Leu				
1555										1560					1565				
Met	Lys	Leu	His	Lys	Asp	Asn	Thr	Thr	Cys	Tyr	Glu	Phe	Lys	Lys	Phe				
1570										1575					1580				
Leu	Leu	Tyr	Ala	Arg	Gln	Met	Glu	Ile	Arg	Gly	Val	Asp	Leu	Asp	Ala				
1585										1590					1595				
Pro	Tyr	Tyr	Asn	Tyr	Ile	Ile	Ser	Phe	Thr	Val	Pro	Asp	Ile	Asp	Asn				
1605										1610					1615				
Val	Thr	Val	Leu	Asp	Tyr	Asp	Ala	Arg	Glu	Gln	Arg	Val	Tyr	Trp	Ser				
1620										1625					1630				
Asp	Val	Arg	Thr	Gln	Ala	Ile	Lys	Arg	Ala	Phe	Ile	Asn	Gly	Thr	Gly				

- 53 -

1635	1640	1645
Val Glu Thr Val Val Ser Ala Asp Leu Pro Asn Ala His Gly Leu Ala		
1650	1655	1660
Val Asp Trp Val Ser Arg Asn Leu Phe Trp Thr Ser Tyr Asp Thr Asn		
1665	1670	1675
Lys Lys Gln Ile Asn Val Ala Arg Leu Asp Gly Ser Phe Lys Asn Ala		1680
1685	1690	1695
Val Val Gln Gly Leu Glu Gln Pro His Gly Leu Val Val His Pro Leu		
1700	1705	1710
Arg Gly Lys Leu Tyr Trp Thr Asp Gly Asp Asn Ile Ser Met Ala Asn		
1715	1720	1725
Met Asp Gly Ser Asn Arg Thr Leu Leu Phe Ser Gly Gln Lys Gly Pro		
1730	1735	1740
Val Gly Leu Ala Ile Asp Phe Pro Glu Ser Lys Leu Tyr Trp Ile Ser		
1745	1750	1755
Ser Gly Asn His Thr Ile Asn Arg Cys Asn Leu Asp Gly Ser Gly Leu		1760
1765	1770	1775
Glu Val Ile Asp Ala Met Arg Ser Gln Leu Gly Lys Ala Thr Ala Leu		
1780	1785	1790
Ala Ile Met Gly Asp Lys Leu Trp Trp Ala Asp Gln Val Ser Glu Lys		
1795	1800	1805
Met Gly Thr Cys Ser Lys Ala Asp Gly Ser Gly Ser Val Val Leu Arg		
1810	1815	1820
Asn Ser Thr Thr Leu Val Met His Met Lys Val Tyr Asp Glu Ser Ile		
1825	1830	1835
Gln Leu Asp His Lys Gly Thr Asn Pro Cys Ser Val Asn Asn Gly Asp		1840
1845	1850	1855
Cys Ser Gln Leu Cys Leu Pro Thr Ser Glu Thr Thr Arg Ser Cys Met		
1860	1865	1870
Cys Thr Ala Gly Tyr Ser Leu Arg Ser Gly Gln Gln Ala Cys Glu Gly		
1875	1880	1885
Val Gly Ser Phe Leu Leu Tyr Ser Val His Glu Gly Ile Arg Gly Ile		
1890	1895	1900
Pro Leu Asp Pro Asn Asp Lys Ser Asp Ala Leu Val Pro Val Ser Gly		
1905	1910	1915
Thr Ser Leu Ala Val Gly Ile Asp Phe His Ala Glu Asn Asp Thr Ile		1920
1925	1930	1935
Tyr Trp Val Asp Met Gly Leu Ser Thr Ile Ser Arg Ala Lys Arg Asp		
1940	1945	1950
Gln Thr Trp Arg Glu Asp Val Val Thr Asn Gly Ile Gly Arg Val Glu		
1955	1960	1965
Gly Ile Ala Val Asp Trp Ile Ala Gly Asn Ile Tyr Trp Thr Asp Gln		
1970	1975	1980
Gly Phe Asp Val Ile Glu Val Ala Arg Leu Asn Gly Ser Phe Arg Tyr		
1985	1990	1995
Val Val Ile Ser Gln Gly Leu Asp Lys Pro Arg Ala Ile Thr Val His		2000
2005	2010	2015
Pro Glu Lys Gly Tyr Leu Phe Trp Thr Glu Trp Gly Gln Tyr Pro Arg		
2020	2025	2030
Ile Glu Arg Ser Arg Leu Asp Gly Thr Glu Arg Val Val Leu Val Asn		
2035	2040	2045
Val Ser Ile Ser Trp Pro Asn Gly Ile Ser Val Asp Tyr Gln Asp Gly		

- 54 -

2050	2055	2060
Lys Leu Tyr Trp Cys Asp Ala Arg Thr Asp Lys Ile Glu Arg Ile Asp		
2065	2070	2075
Leu Glu Thr Gly Glu Asn Arg Glu Val Val Leu Ser Ser Asn Asn Met		2080
	2085	2090
Asp Met Phe Ser Val Ser Val Phe Glu Asp Phe Ile Tyr Trp Ser Asp		2095
	2100	2105
Arg Thr His Ala Asn Gly Ser Ile Lys Arg Gly Ser Lys Asp Asn Ala		2110
	2115	2120
Thr Asp Ser Val Pro Leu Arg Thr Gly Ile Gly Val Gln Leu Lys Asp		2125
	2130	2135
Ile Lys Val Phe Asn Arg Asp Arg Gln Lys Gly Thr Asn Val Cys Ala		2140
2145	2150	2155
Val Ala Asn Gly Gly Cys Gln Gln Leu Cys Leu Tyr Arg Gly Arg Gly		2160
	2165	2170
Gln Arg Ala Cys Ala Cys Ala His Gly Met Leu Ala Glu Asp Gly Ala		2175
	2180	2185
Ser Cys Arg Glu Tyr Ala Gly Tyr Leu Leu Tyr Ser Glu Arg Thr Ile		2190
	2195	2200
Leu Lys Ser Ile His Leu Ser Asp Glu Arg Asn Leu Asn Ala Pro Val		2205
	2210	2215
Gln Pro Phe Glu Asp Pro Glu His Met Lys Asn Val Ile Ala Leu Ala		2220
2225	2230	2235
Phe Asp Tyr Arg Ala Gly Thr Ser Pro Gly Thr Pro Asn Arg Ile Phe		2240
	2245	2250
Phe Ser Asp Ile His Phe Gly Asn Ile Gln Gln Ile Asn Asp Asp Gly		2255
	2260	2265
Ser Arg Arg Ile Thr Ile Val Glu Asn Val Gly Ser Val Glu Gly Leu		2270
	2275	2280
Ala Tyr His Arg Gly Trp Asp Thr Leu Tyr Trp Thr Ser Tyr Thr Thr		2285
	2290	2295
Ser Thr Ile Thr Arg His Thr Val Asp Gln Thr Arg Pro Gly Ala Phe		2300
2305	2310	2315
Glu Arg Glu Thr Val Ile Thr Met Ser Gly Asp Asp His Pro Arg Ala		2320
	2325	2330
Phe Val Leu Asp Glu Cys Gln Asn Leu Met Phe Trp Thr Asn Trp Asn		2335
	2340	2345
Glu Gln His Pro Ser Ile Met Arg Ala Ala Leu Ser Gly Ala Asn Val		2350
	2355	2360
Leu Thr Leu Ile Glu Lys Asp Ile Arg Thr Pro Asn Gly Leu Ala Ile		2365
	2370	2375
Asp His Arg Ala Glu Lys Leu Tyr Phe Ser Asp Ala Thr Leu Asp Lys		2380
2385	2390	2395
Ile Glu Arg Cys Glu Tyr Asp Gly Ser His Arg Tyr Val Ile Leu Lys		2400
	2405	2410
Ser Glu Pro Val His Pro Phe Gly Leu Ala Val Tyr Gly Glu His Ile		2415
	2420	2425
Phe Trp Thr Asp Trp Val Arg Arg Ala Val Gln Arg Ala Asn Lys His		2430
	2435	2440
Val Gly Ser Asn Met Lys Leu Leu Arg Val Asp Ile Pro Gln Gln Pro		2445
	2450	2455
Met Gly Ile Ile Ala Val Ala Asn Asp Thr Asn Ser Cys Glu Leu Ser		2460

- 55 -

2465		2470		2475		2480
Pro Cys Arg Ile Asn Asn Gly Gly Cys Gln Asp Leu Cys Leu Leu Thr						
	2485		2490		2495	
His Gln Gly His Val Asn Cys Ser Cys Arg Gly Gly Arg Ile Leu Gln						
	2500		2505		2510	
Asp Asp Leu Thr Cys Arg Ala Val Asn Ser Ser Cys Arg Ala Gln Asp						
	2515		2520		2525	
Glu Phe Glu Cys Ala Asn Gly Glu Cys Ile Asn Phe Ser Leu Thr Cys						
	2530		2535		2540	
Asp Gly Val Pro His Cys Lys Asp Lys Ser Asp Glu Lys Pro Ser Tyr						
2545		2550		2555		2560
Cys Asn Ser Arg Arg Cys Lys Lys Thr Phe Arg Gln Cys Ser Asn Gly						
	2565		2570		2575	
Arg Cys Val Ser Asn Met Leu Trp Cys Asn Gly Ala Asp Asp Cys Gly						
	2580		2585		2590	
Asp Gly Ser Asp Glu Ile Pro Cys Asn Lys Thr Ala Cys Gly Val Gly						
	2595		2600		2605	
Glu Phe Arg Cys Arg Asp Gly Thr Cys Ile Gly Asn Ser Ser Arg Cys						
	2610		2615		2620	
Asn Gln Phe Val Asp Cys Glu Asp Ala Ser Asp Glu Met Asn Cys Ser						
2625		2630		2635		2640
Ala Thr Asp Cys Ser Ser Tyr Phe Arg Leu Gly Val Lys Gly Val Leu						
	2645		2650		2655	
Phe Gln Pro Cys Glu Arg Thr Ser Leu Cys Tyr Ala Pro Ser Trp Val						
	2660		2665		2670	
Cys Asp Gly Ala Asn Asp Cys Gly Asp Tyr Ser Asp Glu Arg Asp Cys						
	2675		2680		2685	
Pro Gly Val Lys Arg Pro Arg Cys Pro Leu Asn Tyr Phe Ala Cys Pro						
	2690		2695		2700	
Ser Gly Arg Cys Ile Pro Met Ser Trp Thr Cys Asp Lys Glu Asp Asp						
2705		2710		2715		2720
Cys Glu His Gly Glu Asp Glu Thr His Cys Asn Lys Phe Cys Ser Glu						
	2725		2730		2735	
Ala Gln Phe Glu Cys Gln Asn His Arg Cys Ile Ser Lys Gln Trp Leu						
	2740		2745		2750	
Cys Asp Gly Ser Asp Asp Cys Gly Asp Gly Ser Asp Glu Ala Ala His						
	2755		2760		2765	
Cys Glu Gly Lys Thr Cys Gly Pro Ser Ser Phe Ser Cys Pro Gly Thr						
	2770		2775		2780	
His Val Cys Val Pro Glu Arg Trp Leu Cys Asp Gly Asp Lys Asp Cys						
2785		2790		2795		2800
Ala Asp Gly Ala Asp Glu Ser Ile Ala Ala Gly Cys Leu Tyr Asn Ser						
	2805		2810		2815	
Thr Cys Asp Asp Arg Glu Phe Met Cys Gln Asn Arg Gln Cys Ile Pro						
	2820		2825		2830	
Lys His Phe Val Cys Asp His Asp Arg Asp Cys Ala Asp Gly Ser Asp						
	2835		2840		2845	
Glu Ser Pro Glu Cys Glu Tyr Pro Thr Cys Gly Pro Ser Glu Phe Arg						
	2850		2855		2860	
Cys Ala Asn Gly Arg Cys Leu Ser Ser Arg Gln Trp Glu Cys Asp Gly						
2865		2870		2875		2880
Glu Asn Asp Cys His Asp Gln Ser Asp Glu Ala Pro Lys Asn Pro His						

- 56 -

	2885		2890		2895
Cys Thr Ser	Pro Glu His Lys Cys	Asn Ala Ser Ser	Gln Phe Leu Cys		
	2900		2905		2910
Ser Ser Gly	Arg Cys Val Ala Glu	Ala Leu Leu Cys	Asn Gly Gln Asp		
	2915		2920		2925
Asp Cys Gly	Asp Ser Ser Asp	Glu Arg Gly Cys	His Ile Asn Glu Cys		
	2930		2935		2940
Leu Ser Arg	Lys Leu Ser Gly	Cys Ser Gln Asp	Cys Glu Asp Leu Lys		
	2945		2950		2955
Ile Gly Phe	Lys Cys Arg Cys	Arg Pro Gly Phe	Arg Leu Lys Asp Asp		
	2965		2970		2975
Gly Arg Thr	Cys Ala Asp Val	Asp Glu Cys Ser	Thr Thr Phe Pro Cys		
	2980		2985		2990
Ser Gln Arg	Cys Ile Asn Thr	His Gly Ser Tyr	Lys Cys Leu Cys Val		
	2995		3000		3005
Glu Gly Tyr	Ala Pro Arg Gly	Gly Asp Pro His	Ser Cys Lys Ala Val		
	3010		3015		3020
Thr Asp Glu	Glu Pro Phe Leu	Ile Phe Ala Asn	Arg Tyr Tyr Leu Arg		
	3025		3030		3035
Lys Leu Asn	Leu Asp Gly Ser	Asn Tyr Thr Leu	Leu Lys Gln Gly Leu		
	3045		3050		3055
Asn Asn Ala	Val Ala Leu Asp	Phe Asp Tyr Arg	Glu Gln Met Ile Tyr		
	3060		3065		3070
Trp Thr Asp	Val Thr Thr Gln	Gly Ser Met Ile	Arg Arg Met His Leu		
	3075		3080		3085
Asn Gly Ser	Asn Val Gln Val	Leu His Arg Thr	Gly Leu Ser Asn Pro		
	3090		3095		3100
Asp Gly Leu	Ala Val Asp Trp	Val Gly Gly Asn	Leu Tyr Trp Cys Asp		
	3105		3110		3115
Lys Gly Arg	Asp Thr Ile Glu	Val Ser Lys Leu	Asn Gly Ala Tyr Arg		
	3125		3130		3135
Thr Val Leu	Val Ser Ser Gly	Leu Arg Glu Pro	Arg Ala Leu Val Val		
	3140		3145		3150
Asp Val Gln	Asn Gly Tyr Leu	Tyr Trp Thr Asp	Trp Gly Asp His Ser		
	3155		3160		3165
Leu Ile Gly	Arg Ile Gly Met	Asp Gly Ser Ser	Arg Ser Val Ile Val		
	3170		3175		3180
Asp Thr Lys	Ile Thr Trp Pro	Asn Gly Leu Thr	Leu Asp Tyr Val Thr		
	3185		3190		3195
Glu Arg Ile	Tyr Trp Ala Asp	Ala Arg Glu Asp	Tyr Ile Glu Phe Ala		
	3205		3210		3215
Ser Leu Asp	Gly Ser Asn Arg	His Val Val Leu	Ser Gln Asp Ile Pro		
	3220		3225		3230
His Ile Phe	Ala Leu Thr Leu	Phe Glu Asp Tyr	Val Tyr Trp Thr Asp		
	3235		3240		3245
Trp Glu Thr	Lys Ser Ile Asn	Arg Ala His Lys	Thr Thr Gly Thr Asn		
	3250		3255		3260
Lys Thr Leu	Leu Ile Ser Thr	Leu His Arg Pro	Met Asp Leu His Val		
	3265		3270		3275
Phe His Ala	Leu Arg Gln Pro	Asp Val Pro Asn	His Pro Cys Lys Val		
	3285		3290		3295
Asn Asn Gly	Gly Cys Ser Asn	Leu Cys Leu Leu	Ser Pro Gly Gly Gly		

- 57 -

3300	3305	3310
His Lys Cys Ala Cys Pro Thr Asn Phe Tyr Leu Gly Ser Asp Gly Arg		
3315	3320	3325
Thr Cys Val Ser Asn Cys Thr Ala Ser Gln Phe Val Cys Lys Asn Asp		
3330	3335	3340
Lys Cys Ile Pro Phe Trp Trp Lys Cys Asp Thr Glu Asp Asp Cys Gly		
3345	3350	3355
Asp His Ser Asp Glu Pro Pro Asp Cys Pro Glu Phe Lys Cys Arg Pro		
3365	3370	3375
Gly Gln Phe Gln Cys Ser Thr Gly Ile Cys Thr Asn Pro Ala Phe Ile		
3380	3385	3390
Cys Asp Gly Asp Asn Asp Cys Gln Asp Asn Ser Asp Glu Ala Asn Cys		
3395	3400	3405
Asp Ile His Val Cys Leu Pro Ser Gln Phe Lys Cys Thr Asn Thr Asn		
3410	3415	3420
Arg Cys Ile Pro Gly Ile Phe Arg Cys Asn Gly Gln Asp Asn Cys Gly		
3425	3430	3435
Asp Gly Glu Asp Glu Arg Asp Cys Pro Glu Val Thr Cys Ala Pro Asn		
3445	3450	3455
Gln Phe Gln Cys Ser Ile Thr Lys Arg Cys Ile Pro Arg Val Trp Val		
3460	3465	3470
Cys Asp Arg Asp Asn Asp Cys Val Asp Gly Ser Asp Glu Pro Ala Asn		
3475	3480	3485
Cys Thr Gln Met Thr Cys Gly Val Asp Glu Phe Arg Cys Lys Asp Ser		
3490	3495	3500
Gly Arg Cys Ile Pro Ala Arg Trp Lys Cys Asp Gly Glu Asp Asp Cys		
3505	3510	3515
Gly Asp Gly Ser Asp Glu Pro Lys Glu Glu Cys Asp Glu Arg Thr Cys		
3525	3530	3535
Glu Pro Tyr Gln Phe Arg Cys Lys Asn Asn Arg Cys Val Pro Gly Arg		
3540	3545	3550
Trp Gln Cys Asp Tyr Asp Asn Asp Cys Gly Asp Asn Ser Asp Glu Glu		
3555	3560	3565
Ser Cys Thr Pro Arg Pro Cys Ser Glu Ser Glu Phe Ser Cys Ala Asn		
3570	3575	3580
Gly Arg Cys Ile Ala Gly Arg Trp Lys Cys Asp Gly Asp His Asp Cys		
3585	3590	3595
Ala Asp Gly Ser Asp Glu Lys Asp Cys Thr Pro Arg Cys Asp Met Asp		
3605	3610	3615
Gln Phe Gln Cys Lys Ser Gly His Cys Ile Pro Leu Arg Trp Arg Cys		
3620	3625	3630
Asp Ala Asp Ala Asp Cys Met Asp Gly Ser Asp Glu Glu Ala Cys Gly		
3635	3640	3645
Thr Gly Val Arg Thr Cys Pro Leu Asp Glu Phe Gln Cys Asn Asn Thr		
3650	3655	3660
Leu Cys Lys Pro Leu Ala Trp Lys Cys Asp Gly Glu Asp Asp Cys Gly		
3665	3670	3675
Asp Asn Ser Asp Glu Asn Pro Glu Glu Cys Ala Arg Phe Val Cys Pro		
3685	3690	3695
Pro Asn Arg Pro Phe Arg Cys Lys Asn Asp Arg Val Cys Leu Trp Ile		
3700	3705	3710
Gly Arg Gln Cys Asp Gly Thr Asp Asn Cys Gly Asp Gly Thr Asp Glu		

- 58 -

```

3715          3720          3725
Glu Asp Cys Glu Pro Pro Thr Ala His Thr Thr His Cys Lys Asp Lys
3730          3735          3740
Lys Glu Phe Leu Cys Arg Asn Gln Arg Cys Leu Ser Ser Ser Leu Arg
3745          3750          3755          3760
Cys Asn Met Phe Asp Asp Cys Gly Asp Gly Ser Asp Glu Glu Asp Cys
3765          3770          3775
Ser Ile Asp Pro Lys Leu Thr Ser Cys Ala Thr Asn Ala Ser Ile Cys
3780          3785          3790
Gly Asp Glu Ala Arg Cys Val Arg Thr Glu Lys Ala Ala Tyr Cys Ala
3795          3800          3805
Cys Arg Ser Gly Phe His Thr Val Pro Gly Gln Pro Gly Cys Gln Asp
3810          3815          3820
Ile Asn Glu Cys Leu Arg Phe Gly Thr Cys Ser Gln Leu Cys Asn Asn
3825          3830          3835          3840
Thr Lys Gly Gly His Leu Cys Ser Cys Ala Arg Asn Phe Met Lys Thr
3845          3850          3855
His Asn Thr Cys Lys Ala Glu Gly Ser Glu Tyr Gln Val Leu Tyr Ile
3860          3865          3870
Ala Asp Asp Asn Glu Ile Arg Ser Leu Phe Pro Gly His Pro His Ser
3875          3880          3885
Ala Tyr Glu Gln Ala Phe Gln Gly Asp Glu Ser Val Arg Ile Asp Ala
3890          3895          3900
Met Asp Val His Val Lys Ala Gly Arg Val Tyr Trp Thr Asn Trp His
3905          3910          3915          3920
Thr Gly Thr Ile Ser Tyr Arg Ser Leu Pro Pro Ala Ala Pro Pro Thr
3925          3930          3935
Thr Ser Asn Arg His Arg Arg Gln Ile Asp Arg Gly Val Thr His Leu
3940          3945          3950
Asn Ile Ser Gly Leu Lys Met Pro Arg Gly Ile Ala Ile Asp Trp Val
3955          3960          3965
Ala Gly Asn Val Tyr Trp Thr Asp Ser Gly Arg Asp Val Ile Glu Val
3970          3975          3980
Ala Gln Met Lys Gly Glu Asn Arg Lys Thr Leu Ile Ser Gly Met Ile
3985          3990          3995          4000
Asp Glu Pro His Ala Ile Val Val Asp Pro Leu Arg Gly Thr Met Tyr
4005          4010          4015
Trp Ser Asp Trp Gly Asn His Pro Lys Ile Glu Thr Ala Ala Met Asp
4020          4025          4030
Gly Thr Leu Arg Glu Thr Leu Val Gln Asp Asn Ile Gln Trp Pro Thr
4035          4040          4045
Gly Leu Ala Val Asp Tyr His Asn Glu Arg Leu Tyr Trp Ala Asp Ala
4050          4055          4060
Lys Leu Ser Val Ile Gly Ser Ile Arg Leu Asn Gly Thr Asp Pro Ile
4065          4070          4075          4080
Val Ala Ala Asp Ser Lys Arg Gly Leu Ser His Pro Phe Ser Ile Asp
4085          4090          4095
Val Phe Glu Asp Tyr Ile Tyr Gly Val Thr Tyr Ile Asn Asn Arg Val
4100          4105          4110
Phe Lys Ile His Lys Phe Gly His Ser Pro Leu Val Asn Leu Thr Gly
4115          4120          4125
Gly Leu Ser His Ala Ser Asp Val Val Leu Tyr His Gln His Lys Gln

```


- 59 -

4130 4135 4140
 Pro Glu Val Thr Asn Pro Cys Asp Arg Lys Lys Cys Glu Trp Leu Cys
 4145 4150 4155 4160
 Leu Leu Ser Pro Ser Gly Pro Val Cys Thr Cys Pro Asn Gly Lys Arg
 4165 4170 4175
 Leu Asp Asn Gly Thr Cys Val Pro Val Pro Ser Pro Thr Pro Pro Pro
 4180 4185 4190
 Asp Ala Pro Arg Pro Gly Thr Cys Asn Leu Gln Cys Phe Asn Gly Gly
 4195 4200 4205
 Ser Cys Phe Leu Asn Ala Arg Arg Gln Pro Lys Cys Arg Cys Gln Pro
 4210 4215 4220
 Arg Tyr Thr Gly Asp Lys Cys Glu Leu Asp Gln Cys Trp Glu His Cys
 4225 4230 4235 4240
 Arg Asn Gly Gly Thr Cys Ala Ala Ser Pro Ser Gly Met Pro Thr Cys
 4245 4250 4255
 Arg Cys Pro Thr Gly Phe Thr Gly Pro Lys Cys Thr Gln Gln Val Cys
 4260 4265 4270
 Ala Gly Tyr Cys Ala Asn Asn Ser Thr Cys Thr Val Asn Gln Gly Asn
 4275 4280 4285
 Gln Pro Gln Cys Arg Cys Leu Pro Gly Phe Leu Gly Asp Arg Cys Gln
 4290 4295 4300
 Tyr Arg Gln Cys Ser Gly Tyr Cys Glu Asn Phe Gly Thr Cys Gln Met
 4305 4310 4315 4320
 Ala Ala Asp Gly Ser Arg Gln Cys Arg Cys Thr Ala Tyr Phe Glu Gly
 4325 4330 4335
 Ser Arg Cys Glu Val Asn Lys Cys Ser Arg Cys Leu Glu Gly Ala Cys
 4340 4345 4350
 Val Val Asn Lys Gln Ser Gly Asp Val Thr Cys Asn Cys Thr Asp Gly
 4355 4360 4365
 Arg Val Ala Pro Ser Cys Leu Thr Cys Val Gly His Cys Ser Asn Gly
 4370 4375 4380
 Gly Ser Cys Thr Met Asn Ser Lys Met Met Pro Glu Cys Gln Cys Pro
 4385 4390 4395 4400
 Pro His Met Thr Gly Pro Arg Cys Glu Glu His Val Phe Ser Gln Gln
 4405 4410 4415
 Gln Pro Gly His Ile Ala Ser Ile Leu Ile Pro Leu Leu Leu Leu Leu
 4420 4425 4430
 Leu Leu Val Leu Val Ala Gly Val Val Phe Trp Tyr Lys Arg Arg Val
 4435 4440 4445
 Gln Gly Ala Lys Gly Phe Gln His Gln Arg Met Thr Asn Gly Ala Met
 4450 4455 4460
 Asn Val Glu Ile Gly Asn Pro Thr Tyr Lys Met Tyr Glu Gly Gly Glu
 4465 4470 4475 4480
 Pro Asp Asp Val Gly Gly Leu Leu Asp Ala Asp Phe Ala Leu Asp Pro
 4485 4490 4495
 Asp Lys Pro Thr Asn Phe Thr Asn Pro Val Tyr Ala Thr Leu Tyr Met
 4500 4505 4510
 Gly Gly His Gly Ser Arg His Ser Leu Ala Ser Thr Asp Glu Lys Arg
 4515 4520 4525
 Glu Leu Leu Gly Arg Gly Pro Glu Asp Glu Ile Gly Asp Pro Leu Ala
 4530 4535 4540

- 60 -

<210> 11
 <211> 478
 <212> DNA
 <213> Mus musculus

<220>
 <221> misc_feature
 <222> 15
 <223> n is any

<400> 11
 gayatygtsc tsacncagws bccbgccacc ctgtctgtgt ctccaggaga tagcgtcagt
 60
 ctttcctgca gggccagcca aagtatcagc aacaacctac actggtatca acaaaaatca
 120
 catgagtctc caaggattct catcaagtat gcatccagc ccattctatgg gatccccctca
 180
 aggttcagtg gcagtggatc agggacattt ttcaactctca ttgtcaacag tgtggggact
 240
 gaagattttg gaatgtattt ctgtcaacag agtcacagct ggctctctac gttcgggtact
 300
 gggaccaagc tggagctgaa acgggctgat gctgcaccaa ctgtatccat cttccaccac
 360
 tccagtgagc agttaacatc tggaggtgcc tcagtcgtgt gcttcttgaa caacttctac
 420
 ccagagaca tcaatgtcaa gtggaagatt gatggcagtg aacgacaaaa tggcgctcc
 478

<210> 12
 <211> 159
 <212> PRT
 <213> Mus musculus

<400> 12
 Asp Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Val Ser Pro Gly
 1 5 10 15
 Asp Ser Val Ser Leu Ser Cys Arg Ala Ser Gln Ser Ile Ser Asn Asn
 20 25 30
 Leu His Trp Tyr Gln Gln Lys Ser His Glu Ser Pro Arg Ile Leu Ile
 35 40 45
 Lys Tyr Ala Ser Gln Ser Ile Tyr Gly Ile Pro Ser Arg Phe Ser Gly
 50 55 60
 Ser Gly Ser Gly Thr Phe Phe Thr Leu Ile Val Asn Ser Val Gly Thr
 65 70 75 80
 Glu Asp Phe Gly Met Tyr Phe Cys Gln Gln Ser His Ser Trp Pro Leu
 85 90 95
 Thr Phe Gly Thr Gly Thr Lys Leu Glu Leu Lys Arg Ala Asp Ala Ala
 100 105 110
 Pro Thr Val Ser Ile Phe Pro Pro Ser Ser Glu Gln Leu Thr Ser Gly
 115 120 125
 Gly Ala Ser Val Val Cys Phe Leu Asn Asn Phe Tyr Pro Arg Asp Ile
 130 135 140

- 61 -

Asn Val Lys Trp Lys Ile Asp Gly Ser Glu Arg Gln Asn Gly Val
 145 150 155

<210> 13
 <211> 366
 <212> DNA
 <213> Mus musculus

<400> 13
 gaggttaagy tkgttgartc tggaggagac ttagtgaaac ctggagggtc cctgaaactc
 60
 gcctgtgcag cctctggatt cactttcagt aacgatgccca tgtcttgggt tcgccagact
 120
 ccagaaaaga ggctggagtg ggctgcaccc attagtagtg ttggtaacac ctactatcca
 180
 gacagtgtga agggccgatt caccatctcc agagataatg ccaggaacat tctatacctg
 240
 caaatgagta gtgtgaggtc tgaggacacg gccatgtatt actgtgcaag aggctatggt
 300
 gttagtcctt ggttttctta ctggggccaa gggactctag tcaccgtctc ctcagccaaa
 360
 acaaca
 366

<210> 14
 <211> 122
 <212> PRT
 <213> Mus musculus

<400> 14
 Glu Val Lys Leu Val Glu Ser Gly Gly Asp Leu Val Lys Pro Gly Gly
 1 5 10 15
 Ser Leu Lys Leu Ala Cys Ala Ala Ser Gly Phe Thr Phe Ser Asn Asp
 20 25 30
 Ala Met Ser Trp Val Arg Gln Thr Pro Glu Lys Arg Leu Glu Trp Val
 35 40 45
 Ala Ser Ile Ser Ser Val Gly Asn Thr Tyr Tyr Pro Asp Ser Val Lys
 50 55 60
 Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Arg Asn Ile Leu Tyr Leu
 65 70 75 80
 Gln Met Ser Ser Val Arg Ser Glu Asp Thr Ala Met Tyr Tyr Cys Ala
 85 90 95
 Arg Gly Tyr Gly Val Ser Pro Trp Phe Ser Tyr Trp Gly Gln Gly Thr
 100 105 110
 Leu Val Thr Val Ser Ser Ala Lys Thr Thr
 115 120

<210> 15
 <211> 493
 <212> DNA
 <213> Mus musculus

- 62 -

<400> 15
 gaygttytba tgacycarac yccactctcc ctgcctgtca gtcttggaga tcaagcctcc
 60
 atctcttgca gatctagtca gaacattgta catagtagtg gaaacaccta tttagaatgg
 120
 tacctgcaga aaccaggcca gtctccaaag ctctgatct acaaagtctc caaccgattt
 180
 tctgggggtcc cagacaggtt cagtggcagt ggatcagggg cagatttcac actcaagatc
 240
 agcagagtgg aggtcgagga tctgggaatt tattactgct ttcaaggctc acatgttccg
 300
 tacacgttcg gagggggggac caagctggaa ataaaacggg ctgatgctgc accaactgta
 360
 tccatcttcc caccatccag tgagcagtta acatctggag gtgcctcagt cgtgtgcttc
 420
 ttgaacaact tctaccccag agacatcaat gtcaagtgga agattgatgg cagtgaacga
 480
 caaaatggcg tcc
 493

<210> 16
 <211> 164
 <212> PRT
 <213> Mus musculus

<400> 16
 Asp Val Leu Met Thr Gln Thr Pro Leu Ser Leu Pro Val Ser Leu Gly
 1 5 10 15
 Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Asn Ile Val His Ser
 20 25 30
 Ser Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln Ser
 35 40 45
 Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
 50 55 60
 Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
 65 70 75 80
 Ser Arg Val Glu Ala Glu Asp Leu Gly Ile Tyr Tyr Cys Phe Gln Gly
 85 90 95
 Ser His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
 100 105 110
 Arg Ala Asp Ala Ala Pro Thr Val Ser Ile Phe Pro Pro Ser Ser Glu
 115 120 125
 Gln Leu Thr Ser Gly Gly Ala Ser Val Val Cys Phe Leu Asn Asn Phe
 130 135 140
 Tyr Pro Arg Asp Ile Asn Val Lys Trp Lys Ile Asp Gly Ser Glu Arg
 145 150 155 160
 Gln Asn Gly Val

<210> 17
 <211> 354
 <212> DNA

- 63 -

<213> Mus musculus

<400> 17

gaggtyatgy tkgtygartc tggaggaggc ttagtgaagc ctggaggggc cctgaaactc
 60
 tctgtgtag cctctggatt cactttcagt agatatacca tgtcttgggt tcgccagact
 120
 ccggcgaaga gactggagtg ggtcgcaacc atcaattttg gtaatggtaa cacctactat
 180
 cctgacagtg tgaagggccg attcaccatc tccagagaca atgccaggaa caccctgtat
 240
 ctgcaaataa gcagtctgag gtctgaggac acggccatgt attactgtac aagccttaat
 300
 tgggcttact ggggccaaagg gactctgggc accgtctcct cagccaaaac aaca
 354

<210> 18

<211> 118

<212> PRT

<213> Mus musculus

<400> 18

Glu	Val	Met	Leu	Val	Glu	Ser	Gly	Gly	Gly	Leu	Val	Lys	Pro	Gly	Gly		
1				5					10					15			
Ser	Leu	Lys	Leu	Ser	Cys	Val	Ala	Ser	Gly	Phe	Thr	Phe	Ser	Arg	Tyr		
			20					25					30				
Thr	Met	Ser	Trp	Val	Arg	Gln	Thr	Pro	Ala	Lys	Arg	Leu	Glu	Trp	Val		
		35					40					45					
Ala	Thr	Ile	Asn	Phe	Gly	Asn	Gly	Asn	Thr	Tyr	Tyr	Pro	Asp	Ser	Val		
	50				55						60						
Lys	Gly	Arg	Phe	Thr	Ile	Ser	Arg	Asp	Asn	Ala	Arg	Asn	Thr	Leu	Tyr		
65					70					75				80			
Leu	Gln	Met	Ser	Ser	Leu	Arg	Ser	Glu	Asp	Thr	Ala	Met	Tyr	Tyr	Cys		
			85					90					95				
Thr	Ser	Leu	Asn	Trp	Ala	Tyr	Trp	Gly	Gln	Gly	Thr	Leu	Val	Thr	Val		
		100						105					110				
Ser	Ser	Ala	Lys	Thr	Thr												
			115														

<210> 19

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic peptide fragment representing
 Amyloid-beta 35-42 region

<400> 19

Cys Met Val Gly Gly Val Val Ile Ala
 1 5

- 64 -

<210> 20
<211> 13
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic peptide sequence representing
Amyloid-beta 1-12 region

<400> 20
Asp Ala Glu Phe Arg His Asp Ser Gly Tyr Glu Val Cys
1 5 10

<210> 21
<211> 14
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic peptide sequence representing
carboxyl-terminal 13 amino acid peptide of LRP
with amino-terminal cysteine

<400> 21
Cys Gly Arg Gly Pro Glu Asp Glu Ile Gly Asp Pro Leu Ala
1 5 10

<210> 22
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 22
ggacgccatt ttgtcggttca ctgcca
26

<210> 23
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 23
tgttggttttg gctgaggaga cggtga
26

<210> 24

- 65 -

<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<221> misc_feature
<222> 15, 24
<223> N is any

<400> 24
gayatygtsc tsacncagws bccngc
26

<210> 25
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 25
gargtyaagy tbgytgartc yggagg
26

<210> 26
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 26
gaygtyytba tgacycarac ycca
24

<210> 27
<211> 2256
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)...(2256)
<223> APP 751

<300>
<308> Genbank X06989
<309> 2001-11-15

- 66 -

```

<400> 27
atg ctg ccc ggt ttg gca ctg ctc ctg ctg gcc gcc tgg acg gct cgg      48
Met Leu Pro Gly Leu Ala Leu Leu Leu Leu Ala Ala Trp Thr Ala Arg
  1              5              10              15

gcg ctg gag gta ccc act gat ggt aat gct ggc ctg ctg gct gaa ccc      96
Ala Leu Glu Val Pro Thr Asp Gly Asn Ala Gly Leu Leu Ala Glu Pro
              20              25              30

cag att gcc atg ttc tgt ggc aga ctg aac atg cac atg aat gtc cag      144
Gln Ile Ala Met Phe Cys Gly Arg Leu Asn Met His Met Asn Val Gln
              35              40              45

aat ggg aag tgg gat tca gat cca tca ggg acc aaa acc tgc att gat      192
Asn Gly Lys Trp Asp Ser Asp Pro Ser Gly Thr Lys Thr Cys Ile Asp
              50              55              60

acc aag gaa ggc atc ctg cag tat tgc caa gaa gtc tac cct gaa ctg      240
Thr Lys Glu Gly Ile Leu Gln Tyr Cys Gln Glu Val Tyr Pro Glu Leu
              65              70              75              80

cag atc acc aat gtg gta gaa gcc aac caa cca gtg acc atc cag aac      288
Gln Ile Thr Asn Val Val Glu Ala Asn Gln Pro Val Thr Ile Gln Asn
              85              90              95

tgg tgc aag cgg ggc cgc aag cag tgc aag acc cat ccc cac ttt gtg      336
Trp Cys Lys Arg Gly Arg Lys Gln Cys Lys Thr His Pro His Phe Val
              100              105              110

att ccc tac cgc tgc tta gtt ggt gag ttt gta agt gat gcc ctt ctc      384
Ile Pro Tyr Arg Cys Leu Val Gly Glu Phe Val Ser Asp Ala Leu Leu
              115              120              125

gtt cct gac aag tgc aaa ttc tta cac cag gag agg atg gat gtt tgc      432
Val Pro Asp Lys Cys Lys Phe Leu His Gln Glu Arg Met Asp Val Cys
              130              135              140

gaa act cat ctt cac tgg cac acc gtc gcc aaa gag aca tgc agt gag      480
Glu Thr His Leu His Trp His Thr Val Ala Lys Glu Thr Cys Ser Glu
              145              150              155              160

aag agt acc aac ttg cat gac tac ggc atg ttg ctg ccc tgc gga att      528
Lys Ser Thr Asn Leu His Asp Tyr Gly Met Leu Leu Pro Cys Gly Ile
              165              170              175

gac aag ttc cga ggg gta gag ttt gtg tgt tgc cca ctg gct gaa gaa      576
Asp Lys Phe Arg Gly Val Glu Phe Val Cys Cys Pro Leu Ala Glu Glu
              180              185              190

agt gac aat gtg gat tct gct gat gcg gag gag gat gac tcg gat gtc      624
Ser Asp Asn Val Asp Ser Ala Asp Ala Glu Glu Asp Asp Ser Asp Val

```


- 67 -

195	200	205	
tgg tgg ggc gga gca gac	aca gac tat gca gat	ggg agt gaa gac aaa	672
Trp Trp Gly Gly Ala Asp	Thr Asp Tyr Ala Asp	Gly Ser Glu Asp Lys	
210	215	220	
gta gta gaa gta gca gag	gag gaa gaa gtg gct	gag gtg gaa gaa gaa	720
Val Val Glu Val Ala Glu	Glu Glu Glu Val Ala	Glu Val Glu Glu Glu	
225	230	235 240	
gaa gcc gat gat gac gag	gac gat gag gat ggt	gat gag gta gag gaa	768
Glu Ala Asp Asp Asp	Glu Asp Asp Glu Asp	Gly Asp Glu Val Glu	
245	250	255	
gag gct gag gaa ccc tac	gaa gaa gcc aca gag	aga acc acc agc att	816
Glu Ala Glu Glu Pro Tyr	Glu Glu Ala Thr Glu	Arg Thr Thr Ser Ile	
260	265	270	
gcc acc acc acc acc acc	acc aca gag tct gtg	gaa gag gtg gtt cga	864
Ala Thr Thr Thr Thr Thr	Thr Thr Thr Glu Ser	Val Glu Glu Val Val	
275	280	285	
gag gtg tgc tct gaa caa	gcc gag acg ggg ccg	tgc cga gca atg atc	912
Glu Val Cys Ser Glu Gln	Ala Glu Thr Gly Pro	Cys Arg Ala Met Ile	
290	295	300	
tcc cgc tgg tac ttt gat	gtg act gaa ggg aag	tgt gcc cca ttc ttt	960
Ser Arg Trp Tyr Phe Asp	Val Thr Glu Gly Lys	Cys Ala Pro Phe Phe	
305	310	315 320	
tac ggc gga tgt ggc ggc	aac cgg aac aac ttt	gac aca gaa gag tac	1008
Tyr Gly Gly Cys Gly Gly	Asn Arg Asn Asn Phe	Asp Thr Glu Glu Tyr	
325	330	335	
tgc atg gcc gtg tgt ggc	agc gcc att cct aca	aca gca gcc agt acc	1056
Cys Met Ala Val Cys Gly	Ser Ala Ile Pro Thr	Thr Ala Ala Ser Thr	
340	345	350	
cct gat gcc gtt gac aag	tat ctc gag aca cct	ggg gat gag aat gaa	1104
Pro Asp Ala Val Asp Lys	Tyr Leu Glu Thr Pro	Gly Asp Glu Asn Glu	
355	360	365	
cat gcc cat ttc cag aaa	gcc aaa gag agg ctt	gag gcc aag cac cga	1152
His Ala His Phe Gln Lys	Ala Lys Glu Arg Leu	Glu Ala Lys His Arg	
370	375	380	
gag aga atg tcc cag gtc	atg aga gaa tgg gaa	gag gca gaa cgt caa	1200
Glu Arg Met Ser Gln Val	Met Arg Glu Trp Glu	Glu Ala Glu Arg Gln	
385	390	395 400	
gca aag aac ttg cct aaa	gct gat aag aag gca	gtt atc cag cat ttc	1248
Ala Lys Asn Leu Pro Lys	Ala Asp Lys Lys Ala	Val Ile Gln His Phe	

- 68 -

405	410	415	
cag gag aaa gtg gaa tct ttg gaa	cag gaa gca gcc aac gag aga cag		1296
Gln Glu Lys Val Glu Ser Leu Glu	Gln Glu Ala Ala Asn Glu Arg Gln		
420	425	430	
cag ctg gtg gag aca cac atg gcc aga gtg gaa gcc atg ctc aat gac			1344
Gln Leu Val Glu Thr His Met Ala Arg Val Glu Ala Met Leu Asn Asp			
435	440	445	
cgc cgc cgc ctg gcc ctg gag aac tac atc acc gct ctg cag gct gtt			1392
Arg Arg Arg Leu Ala Leu Glu Asn Tyr Ile Thr Ala Leu Gln Ala Val			
450	455	460	
cct cct cgg cct cgt cac gtg ttc aat atg cta aag aag tat gtc cgc			1440
Pro Pro Arg Pro Arg His Val Phe Asn Met Leu Lys Lys Tyr Val Arg			
465	470	475	480
gca gaa cag aag gac aga cag cac acc cta aag cat ttc gag cat gtg			1488
Ala Glu Gln Lys Asp Arg Gln His Thr Leu Lys His Phe Glu His Val			
485	490	495	
cgc atg gtg gat ccc aag aaa gcc gct cag atc cgg tcc cag gtt atg			1536
Arg Met Val Asp Pro Lys Lys Ala Ala Gln Ile Arg Ser Gln Val Met			
500	505	510	
aca cac ctc cgt gtg att tat gag cgc atg aat cag tct ctc tcc ctg			1584
Thr His Leu Arg Val Ile Tyr Glu Arg Met Asn Gln Ser Leu Ser Leu			
515	520	525	
ctc tac aac gtg cct gca gtg gcc gag gag att cag gat gaa gtt gat			1632
Leu Tyr Asn Val Pro Ala Val Ala Glu Glu Ile Gln Asp Glu Val Asp			
530	535	540	
gag ctg ctt cag aaa gag caa aac tat tca gat gac gtc ttg gcc aac			1680
Glu Leu Leu Gln Lys Glu Gln Asn Tyr Ser Asp Asp Val Leu Ala Asn			
545	550	555	560
atg att agt gaa cca agg atc agt tac gga aac gat gct ctc atg cca			1728
Met Ile Ser Glu Pro Arg Ile Ser Tyr Gly Asn Asp Ala Leu Met Pro			
565	570	575	
tct ttg acc gaa acg aaa acc acc gtg gag ctc ctt ccc gtg aat gga			1776
Ser Leu Thr Glu Thr Lys Thr Thr Val Glu Leu Leu Pro Val Asn Gly			
580	585	590	
gag ttc agc ctg gac gat ctc cag ccg tgg cat tct ttt ggg gct gac			1824
Glu Phe Ser Leu Asp Asp Leu Gln Pro Trp His Ser Phe Gly Ala Asp			
595	600	605	
tct gtg cca gcc aac aca gaa aac gaa gtt gag cct gtt gat gcc cgc			1872
Ser Val Pro Ala Asn Thr Glu Asn Glu Val Glu Pro Val Asp Ala Arg			

- 69 -

610	615	620	
cct gct gcc gac cga gga ctg acc act cga cca ggt tct ggg ttg aca			1920
Pro Ala Ala Asp Arg Gly Leu Thr Thr Arg Pro Gly Ser Gly Leu Thr			
625	630	635	640
aat atc aag acg gag gag atc tct gaa gtg aag atg gat gca gaa ttc			1968
Asn Ile Lys Thr Glu Glu Ile Ser Glu Val Lys Met Asp Ala Glu Phe			
645	650		655
cga cat gac tca gga tat gaa gtt cat cat caa aaa ttg gtg ttc ttt			2016
Arg His Asp Ser Gly Tyr Glu Val His His Gln Lys Leu Val Phe Phe			
660	665		670
gca gaa gat gtg ggt tca aac aaa ggt gca atc att gga ctc atg gtg			2064
Ala Glu Asp Val Gly Ser Asn Lys Gly Ala Ile Ile Gly Leu Met Val			
675	680		685
ggc ggt gtt gtc ata gcg aca gtg atc gtc atc acc ttg gtg atg ctg			2112
Gly Gly Val Val Ile Ala Thr Val Ile Val Ile Thr Leu Val Met Leu			
690	695		700
aag aag aaa cag tac aca tcc att cat cat ggt gtg gtg gag gtt gac			2160
Lys Lys Lys Gln Tyr Thr Ser Ile His His Gly Val Val Glu Val Asp			
705	710	715	720
gcc gct gtc acc cca gag gag cgc cac ctg tcc aag atg cag cag aac			2208
Ala Ala Val Thr Pro Glu Glu Arg His Leu Ser Lys Met Gln Gln Asn			
725	730		735
ggc tac gaa aat cca acc tac aag ttc ttt gag cag atg cag aac tag			2256
Gly Tyr Glu Asn Pro Thr Tyr Lys Phe Phe Glu Gln Met Gln Asn			
740	745		750

<210> 28

<211> 751

<212> PRT

<213> Homo sapiens

<300>

<308> Genbank CAA30050

<309> 2001-11-15

<400> 28

Met Leu Pro Gly Leu Ala Leu Leu Leu Leu Ala Ala Trp Thr Ala Arg			
1	5	10	15
Ala Leu Glu Val Pro Thr Asp Gly Asn Ala Gly Leu Leu Ala Glu Pro			
20	25		30
Gln Ile Ala Met Phe Cys Gly Arg Leu Asn Met His Met Asn Val Gln			
35	40		45

- 70 -

Asn	Gly	Lys	Trp	Asp	Ser	Asp	Pro	Ser	Gly	Thr	Lys	Thr	Cys	Ile	Asp
50						55					60				
Thr	Lys	Glu	Gly	Ile	Leu	Gln	Tyr	Cys	Gln	Glu	Val	Tyr	Pro	Glu	Leu
65					70					75				80	
Gln	Ile	Thr	Asn	Val	Val	Glu	Ala	Asn	Gln	Pro	Val	Thr	Ile	Gln	Asn
			85						90					95	
Trp	Cys	Lys	Arg	Gly	Arg	Lys	Gln	Cys	Lys	Thr	His	Pro	His	Phe	Val
			100					105					110		
Ile	Pro	Tyr	Arg	Cys	Leu	Val	Gly	Glu	Phe	Val	Ser	Asp	Ala	Leu	Leu
		115					120					125			
Val	Pro	Asp	Lys	Cys	Lys	Phe	Leu	His	Gln	Glu	Arg	Met	Asp	Val	Cys
		130				135					140				
Glu	Thr	His	Leu	His	Trp	His	Thr	Val	Ala	Lys	Glu	Thr	Cys	Ser	Glu
145					150					155				160	
Lys	Ser	Thr	Asn	Leu	His	Asp	Tyr	Gly	Met	Leu	Leu	Pro	Cys	Gly	Ile
			165					170						175	
Asp	Lys	Phe	Arg	Gly	Val	Glu	Phe	Val	Cys	Cys	Pro	Leu	Ala	Glu	Glu
			180					185					190		
Ser	Asp	Asn	Val	Asp	Ser	Ala	Asp	Ala	Glu	Glu	Asp	Asp	Ser	Asp	Val
		195					200					205			
Trp	Trp	Gly	Gly	Ala	Asp	Thr	Asp	Tyr	Ala	Asp	Gly	Ser	Glu	Asp	Lys
		210				215					220				
Val	Val	Glu	Val	Ala	Glu	Glu	Glu	Glu	Val	Ala	Glu	Val	Glu	Glu	Glu
225					230					235				240	
Glu	Ala	Asp	Asp	Asp	Glu	Asp	Asp	Glu	Asp	Gly	Asp	Glu	Val	Glu	Glu
			245					250						255	
Glu	Ala	Glu	Glu	Pro	Tyr	Glu	Glu	Ala	Thr	Glu	Arg	Thr	Thr	Ser	Ile
			260					265					270		
Ala	Thr	Thr	Thr	Thr	Thr	Thr	Thr	Glu	Ser	Val	Glu	Glu	Val	Val	Arg
		275					280					285			
Glu	Val	Cys	Ser	Glu	Gln	Ala	Glu	Thr	Gly	Pro	Cys	Arg	Ala	Met	Ile
		290				295					300				
Ser	Arg	Trp	Tyr	Phe	Asp	Val	Thr	Glu	Gly	Lys	Cys	Ala	Pro	Phe	Phe
305					310					315				320	
Tyr	Gly	Gly	Cys	Gly	Gly	Asn	Arg	Asn	Asn	Phe	Asp	Thr	Glu	Glu	Tyr
			325						330					335	
Cys	Met	Ala	Val	Cys	Gly	Ser	Ala	Ile	Pro	Thr	Thr	Ala	Ala	Ser	Thr
			340					345					350		
Pro	Asp	Ala	Val	Asp	Lys	Tyr	Leu	Glu	Thr	Pro	Gly	Asp	Glu	Asn	Glu
		355					360					365			
His	Ala	His	Phe	Gln	Lys	Ala	Lys	Glu	Arg	Leu	Glu	Ala	Lys	His	Arg
		370				375					380				
Glu	Arg	Met	Ser	Gln	Val	Met	Arg	Glu	Trp	Glu	Glu	Ala	Glu	Arg	Gln
385					390					395				400	
Ala	Lys	Asn	Leu	Pro	Lys	Ala	Asp	Lys	Lys	Ala	Val	Ile	Gln	His	Phe
			405						410					415	
Gln	Glu	Lys	Val	Glu	Ser	Leu	Glu	Gln	Glu	Ala	Ala	Asn	Glu	Arg	Gln
			420					425					430		
Gln	Leu	Val	Glu	Thr	His	Met	Ala	Arg	Val	Glu	Ala	Met	Leu	Asn	Asp
		435					440					445			
Arg	Arg	Arg	Leu	Ala	Leu	Glu	Asn	Tyr	Ile	Thr	Ala	Leu	Gln	Ala	Val
		450				455					460				

- 71 -

```

Pro Pro Arg Pro Arg His Val Phe Asn Met Leu Lys Lys Tyr Val Arg
465          470          475          480
Ala Glu Gln Lys Asp Arg Gln His Thr Leu Lys His Phe Glu His Val
          485          490          495
Arg Met Val Asp Pro Lys Lys Ala Ala Gln Ile Arg Ser Gln Val Met
          500          505          510
Thr His Leu Arg Val Ile Tyr Glu Arg Met Asn Gln Ser Leu Ser Leu
          515          520          525
Leu Tyr Asn Val Pro Ala Val Ala Glu Glu Ile Gln Asp Glu Val Asp
          530          535          540
Glu Leu Leu Gln Lys Glu Gln Asn Tyr Ser Asp Asp Val Leu Ala Asn
545          550          555          560
Met Ile Ser Glu Pro Arg Ile Ser Tyr Gly Asn Asp Ala Leu Met Pro
          565          570          575
Ser Leu Thr Glu Thr Lys Thr Thr Val Glu Leu Leu Pro Val Asn Gly
          580          585          590
Glu Phe Ser Leu Asp Asp Leu Gln Pro Trp His Ser Phe Gly Ala Asp
          595          600          605
Ser Val Pro Ala Asn Thr Glu Asn Glu Val Glu Pro Val Asp Ala Arg
          610          615          620
Pro Ala Ala Asp Arg Gly Leu Thr Thr Arg Pro Gly Ser Gly Leu Thr
625          630          635          640
Asn Ile Lys Thr Glu Glu Ile Ser Glu Val Lys Met Asp Ala Glu Phe
          645          650          655
Arg His Asp Ser Gly Tyr Glu Val His His Gln Lys Leu Val Phe Phe
          660          665          670
Ala Glu Asp Val Gly Ser Asn Lys Gly Ala Ile Ile Gly Leu Met Val
          675          680          685
Gly Gly Val Val Ile Ala Thr Val Ile Val Ile Thr Leu Val Met Leu
          690          695          700
Lys Lys Lys Gln Tyr Thr Ser Ile His His Gly Val Val Glu Val Asp
705          710          715          720
Ala Ala Val Thr Pro Glu Glu Arg His Leu Ser Lys Met Gln Gln Asn
          725          730          735
Gly Tyr Glu Asn Pro Thr Tyr Lys Phe Phe Glu Gln Met Gln Asn
          740          745          750

```

<210> 29
 <211> 2088
 <212> DNA
 <213> Homo Sapiens

<220>
 <221> CDS
 <222> (1)...(2088)
 <223> APP 695

<300>
 <308> Genbank Y00264
 <309> 1993-09-12

- 72 -

```

<400> 29
atg ctg ccc ggt ttg gca ctg ctc ctg ctg gcc gcc tgg acg gct cgg      48
Met Leu Pro Gly Leu Ala Leu Leu Leu Leu Ala Ala Trp Thr Ala Arg
  1              5              10              15

gcg ctg gag gta ccc act gat ggt aat gct ggc ctg ctg gct gaa ccc      96
Ala Leu Glu Val Pro Thr Asp Gly Asn Ala Gly Leu Leu Ala Glu Pro
      20              25              30

cag att gcc atg ttc tgt ggc aga ctg aac atg cac atg aat gtc cag     144
Gln Ile Ala Met Phe Cys Gly Arg Leu Asn Met His Met Asn Val Gln
      35              40              45

aat ggg aag tgg gat tca gat cca tca ggg acc aaa acc tgc att gat     192
Asn Gly Lys Trp Asp Ser Asp Pro Ser Gly Thr Lys Thr Cys Ile Asp
      50              55              60

acc aag gaa ggc atc ctg cag tat tgc caa gaa gtc tac cct gaa ctg     240
Thr Lys Glu Gly Ile Leu Gln Tyr Cys Gln Glu Val Tyr Pro Glu Leu
      65              70              75              80

cag atc acc aat gtg gta gaa gcc aac caa cca gtg acc atc cag aac     288
Gln Ile Thr Asn Val Val Glu Ala Asn Gln Pro Val Thr Ile Gln Asn
      85              90              95

tgg tgc aag cgg ggc cgc aag cag tgc aag acc cat ccc cac ttt gtg     336
Trp Cys Lys Arg Gly Arg Lys Gln Cys Lys Thr His Pro His Phe Val
      100             105             110

att ccc tac cgc tgc tta gtt ggt gag ttt gta agt gat gcc ctt ctc     384
Ile Pro Tyr Arg Cys Leu Val Gly Glu Phe Val Ser Asp Ala Leu Leu
      115             120             125

gtt cct gac aag tgc aaa ttc tta cac cag gag agg atg gat gtt tgc     432
Val Pro Asp Lys Cys Lys Phe Leu His Gln Glu Arg Met Asp Val Cys
      130             135             140

gaa act cat ctt cac tgg cac acc gtc gcc aaa gag aca tgc agt gag     480
Glu Thr His Leu His Trp His Thr Val Ala Lys Glu Thr Cys Ser Glu
      145             150             155             160

aag agt acc aac ttg cat gac tac ggc atg ttg ctg ccc tgc gga att     528
Lys Ser Thr Asn Leu His Asp Tyr Gly Met Leu Leu Pro Cys Gly Ile
      165             170             175

gac aag ttc cga ggg gta gag ttt gtg tgt tgc cca ctg gct gaa gaa     576
Asp Lys Phe Arg Gly Val Glu Phe Val Cys Cys Pro Leu Ala Glu Glu
      180             185             190

agt gac aat gtg gat tct gct gat gcg gag gag gat gac tcg gat gtc     624
Ser Asp Asn Val Asp Ser Ala Asp Ala Glu Glu Asp Asp Ser Asp Val
      195             200             205

```

- 73 -

tgg tgg ggc gga gca gac aca gac tat gca gat ggg agt gaa gac aaa	672
Trp Trp Gly Gly Ala Asp Thr Asp Tyr Ala Asp Gly Ser Glu Asp Lys	
210 215 220	
gta gta gaa gta gca gag gag gaa gaa gtg gct gag gtg gaa gaa gaa	720
Val Val Glu Val Ala Glu Glu Glu Glu Val Ala Glu Val Glu Glu Glu	
225 230 235 240	
gaa gcc gat gat gac gag gac gat gag gat ggt gat gag gta gag gaa	768
Glu Ala Asp Asp Asp Glu Asp Asp Glu Asp Gly Asp Glu Val Glu Glu	
245 250 255	
gag gct gag gaa ccc tac gaa gaa gcc aca gag aga acc acc agc att	816
Glu Ala Glu Glu Pro Tyr Glu Glu Ala Thr Glu Arg Thr Thr Ser Ile	
260 265 270	
gcc acc acc acc acc acc acc aca gag tct gtg gaa gag gtg gtt cga	864
Ala Thr Thr Thr Thr Thr Thr Thr Glu Ser Val Glu Glu Val Val Arg	
275 280 285	
gtt cct aca aca gca gcc agt acc cct gat gcc gtt gac aag tat ctc	912
Val Pro Thr Thr Ala Ala Ser Thr Pro Asp Ala Val Asp Lys Tyr Leu	
290 295 300	
gag aca cct ggg gat gag aat gaa cat gcc cat ttc cag aaa gcc aaa	960
Glu Thr Pro Gly Asp Glu Asn Glu His Ala His Phe Gln Lys Ala Lys	
305 310 315 320	
gag agg ctt gag gcc aag cac cga gag aga atg tcc cag gtc atg aga	1008
Glu Arg Leu Glu Ala Lys His Arg Glu Arg Met Ser Gln Val Met Arg	
325 330 335	
gaa tgg gaa gag gca gaa cgt caa gca aag aac ttg cct aaa gct gat	1056
Glu Trp Glu Glu Ala Glu Arg Gln Ala Lys Asn Leu Pro Lys Ala Asp	
340 345 350	
aag aag gca gtt atc cag cat ttc cag gag aaa gtg gaa tct ttg gaa	1104
Lys Lys Ala Val Ile Gln His Phe Gln Glu Lys Val Glu Ser Leu Glu	
355 360 365	
cag gaa gca gcc aac gag aga cag cag ctg gtg gag aca cac atg gcc	1152
Gln Glu Ala Ala Asn Glu Arg Gln Gln Leu Val Glu Thr His Met Ala	
370 375 380	
aga gtg gaa gcc atg ctc aat gac cgc cgc cgc ctg gcc ctg gag aac	1200
Arg Val Glu Ala Met Leu Asn Asp Arg Arg Arg Leu Ala Leu Glu Asn	
385 390 395 400	
tac atc acc gct ctg cag gct gtt cct cct cgg cct cgt cac gtg ttc	1248
Tyr Ile Thr Ala Leu Gln Ala Val Pro Arg Pro Arg His Val Phe	
405 410 415	

- 74 -

aat atg cta aag aag tat gtc cgc gca gaa cag aag gac aga cag cac	1296
Asn Met Leu Lys Lys Tyr Val Arg Ala Glu Gln Lys Asp Arg Gln His	
420 425 430	
acc cta aag cat ttc gag cat gtg cgc atg gtg gat ccc aag aaa gcc	1344
Thr Leu Lys His Phe Glu His Val Arg Met Val Asp Pro Lys Lys Ala	
435 440 445	
gct cag atc cgg tcc cag gtt atg aca cac ctc cgt gtg att tat gag	1392
Ala Gln Ile Arg Ser Gln Val Met Thr His Leu Arg Val Ile Tyr Glu	
450 455 460	
cgc atg aat cag tct ctc tcc ctg ctc tac aac gtg cct gca gtg gcc	1440
Arg Met Asn Gln Ser Leu Ser Leu Leu Tyr Asn Val Pro Ala Val Ala	
465 470 475 480	
gag gag att cag gat gaa gtt gat gag ctg ctt cag aaa gag caa aac	1488
Glu Glu Ile Gln Asp Glu Val Asp Glu Leu Leu Gln Lys Glu Gln Asn	
485 490 495	
tat tca gat gac gtc ttg gcc aac atg att agt gaa cca agg atc agt	1536
Tyr Ser Asp Asp Val Leu Ala Asn Met Ile Ser Glu Pro Arg Ile Ser	
500 505 510	
tac gga aac gat gct ctc atg cca tct ttg acc gaa acg aaa acc acc	1584
Tyr Gly Asn Asp Ala Leu Met Pro Ser Leu Thr Glu Thr Lys Thr Thr	
515 520 525	
gtg gag ctc ctt ccc gtg aat gga gag ttc agc ctg gac gat ctc cag	1632
Val Glu Leu Leu Pro Val Asn Gly Glu Phe Ser Leu Asp Asp Leu Gln	
530 535 540	
ccg tgg cat tct ttt ggg gct gac tct gtg cca gcc aac aca gaa aac	1680
Pro Trp His Ser Phe Gly Ala Asp Ser Val Pro Ala Asn Thr Glu Asn	
545 550 555 560	
gaa gtt gag cct gtt gat gcc cgc cct gct gcc gac cga gga ctg acc	1728
Glu Val Glu Pro Val Asp Ala Arg Pro Ala Ala Asp Arg Gly Leu Thr	
565 570 575	
act cga cca ggt tct ggg ttg aca aat atc aag acg gag gag atc tct	1776
Thr Arg Pro Gly Ser Gly Leu Thr Asn Ile Lys Thr Glu Glu Ile Ser	
580 585 590	
gaa gtg aag atg gat gca gaa ttc cga cat gac tca gga tat gaa gtt	1824
Glu Val Lys Met Asp Ala Glu Phe Arg His Asp Ser Gly Tyr Glu Val	
595 600 605	
cat cat caa aaa ttg gtg ttc ttt gca gaa gat gtg ggt tca aac aaa	1872
His His Gln Lys Leu Val Phe Phe Ala Glu Asp Val Gly Ser Asn Lys	
610 615 620	

- 75 -

ggt gca atc att gga ctc atg gtg ggc ggt gtt gtc ata gcg aca gtg 1920
 Gly Ala Ile Ile Gly Leu Met Val Gly Gly Val Val Ile Ala Thr Val
 625 630 635 640

atc gtc atc acc ttg gtg atg ctg aag aag aaa cag tac aca tcc att 1968
 Ile Val Ile Thr Leu Val Met Leu Lys Lys Lys Gln Tyr Thr Ser Ile
 645 650 655

cat cat ggt gtg gtg gag gtt gac gcc gct gtc acc cca gag gag cgc 2016
 His His Gly Val Val Glu Val Asp Ala Ala Val Thr Pro Glu Glu Arg
 660 665 670

cac ctg tcc aag atg cag cag aac ggc tac gaa aat cca acc tac aag 2064
 His Leu Ser Lys Met Gln Gln Asn Gly Tyr Glu Asn Pro Thr Tyr Lys
 675 680 685

ttc ttt gag cag atg cag aac tag 2088
 Phe Phe Glu Gln Met Gln Asn
 690 695

<210> 30
 <211> 695
 <212> PRT
 <213> Homo Sapiens

<300>
 <308> Genbank CAA68374
 <309> 1993-09-12

<400> 30
 Met Leu Pro Gly Leu Ala Leu Leu Leu Leu Ala Ala Trp Thr Ala Arg
 1 5 10 15
 Ala Leu Glu Val Pro Thr Asp Gly Asn Ala Gly Leu Leu Ala Glu Pro
 20 25 30
 Gln Ile Ala Met Phe Cys Gly Arg Leu Asn Met His Met Asn Val Gln
 35 40 45
 Asn Gly Lys Trp Asp Ser Asp Pro Ser Gly Thr Lys Thr Cys Ile Asp
 50 55 60
 Thr Lys Glu Gly Ile Leu Gln Tyr Cys Gln Glu Val Tyr Pro Glu Leu
 65 70 75 80
 Gln Ile Thr Asn Val Val Glu Ala Asn Gln Pro Val Thr Ile Gln Asn
 85 90 95
 Trp Cys Lys Arg Gly Arg Lys Gln Cys Lys Thr His Pro His Phe Val
 100 105 110
 Ile Pro Tyr Arg Cys Leu Val Gly Glu Phe Val Ser Asp Ala Leu Leu
 115 120 125
 Val Pro Asp Lys Cys Lys Phe Leu His Gln Glu Arg Met Asp Val Cys
 130 135 140
 Glu Thr His Leu His Trp His Thr Val Ala Lys Glu Thr Cys Ser Glu
 145 150 155 160

- 77 -

```

Thr Arg Pro Gly Ser Gly Leu Thr Asn Ile Lys Thr Glu Glu Ile Ser
      580                      585                      590
Glu Val Lys Met Asp Ala Glu Phe Arg His Asp Ser Gly Tyr Glu Val
      595                      600                      605
His His Gln Lys Leu Val Phe Phe Ala Glu Asp Val Gly Ser Asn Lys
      610                      615                      620
Gly Ala Ile Ile Gly Leu Met Val Gly Gly Val Val Ile Ala Thr Val
      625                      630                      635                      640
Ile Val Ile Thr Leu Val Met Leu Lys Lys Lys Gln Tyr Thr Ser Ile
      645                      650                      655
His His Gly Val Val Glu Val Asp Ala Ala Val Thr Pro Glu Glu Arg
      660                      665                      670
His Leu Ser Lys Met Gln Gln Asn Gly Tyr Glu Asn Pro Thr Tyr Lys
      675                      680                      685
Phe Phe Glu Gln Met Gln Asn
      690                      695

```

```

<210> 31
<211> 7671
<212> DNA
<213> Homo Sapiens

```

```

<220>
<221> CDS
<222> (1)...(7671)
<223> NOTCH 1

```

```

<300>
<308> Genbank AF308602
<309> 2000-11-22

```

```

<400> 31
atg ccg ccg ctc ctg gcg ccc ctg ctc tgc ctg gcg ctg ctg ccc gcg 48
Met Pro Pro Leu Leu Ala Pro Leu Leu Cys Leu Ala Leu Leu Pro Ala
1 5 10 15

ctc gcc gca cga ggc ccg cga tgc tcc cag ccc ggt gag acc tgc ctg 96
Leu Ala Ala Arg Gly Pro Arg Cys Ser Gln Pro Gly Glu Thr Cys Leu
20 25 30

aat ggc ggg aag tgt gaa gcg gcc aat ggc acg gag gcc tgc gtc tgt 144
Asn Gly Gly Lys Cys Glu Ala Ala Asn Gly Thr Glu Ala Cys Val Cys
35 40 45

ggc ggg gcc ttc gtg ggc ccg cga tgc cag gac ccc aac ccg tgc ctc 192
Gly Gly Ala Phe Val Gly Pro Arg Cys Gln Asp Pro Asn Pro Cys Leu
50 55 60

agc acc ccc tgc aag aac gcc ggg aca tgc cac gtg gtg gac cgc aga 240
Ser Thr Pro Cys Lys Asn Ala Gly Thr Cys His Val Val Asp Arg Arg
65 70 75 80

```

- 78 -

ggc gtg gca gac tat gcc tgc agc tgt gcc ctg ggc ttc tct ggg ccc	288
Gly Val Ala Asp Tyr Ala Cys Ser Cys Ala Leu Gly Phe Ser Gly Pro	
85 90 95	
ctc tgc ctg aca ccc ctg gac aac gcc tgc ctc acc aac ccc tgc cgc	336
Leu Cys Leu Thr Pro Leu Asp Asn Ala Cys Leu Thr Asn Pro Cys Arg	
100 105 110	
aac ggg ggc acc tgc gac ctg ctc acg ctg acg gag tac aag tgc cgc	384
Asn Gly Gly Thr Cys Asp Leu Leu Thr Leu Thr Glu Tyr Lys Cys Arg	
115 120 125	
tgc ccg ccc ggc tgg tca ggg aaa tcg tgc cag cag gct gac ccg tgc	432
Cys Pro Pro Gly Trp Ser Gly Lys Ser Cys Gln Gln Ala Asp Pro Cys	
130 135 140	
gcc tcc aac ccc tgc gcc aac ggt ggc cag tgc ctg ccc ttc gag gcc	480
Ala Ser Asn Pro Cys Ala Asn Gly Gly Gln Cys Leu Pro Phe Glu Ala	
145 150 155 160	
tcc tac atc tgc cac tgc cca ccc agc ttc cat ggc ccc acc tgc cgg	528
Ser Tyr Ile Cys His Cys Pro Pro Ser Phe His Gly Pro Thr Cys Arg	
165 170 175	
cag gat gtc aac gag tgt ggc cag aag ccc agg ctt tgc cgc cac gga	576
Gln Asp Val Asn Glu Cys Gly Gln Lys Pro Arg Leu Cys Arg His Gly	
180 185 190	
ggc acc tgc cac aac gag gtc ggc tcc tac cgc tgc gtc tgc cgc gcc	624
Gly Thr Cys His Asn Glu Val Gly Ser Tyr Arg Cys Val Cys Arg Ala	
195 200 205	
acc cac act ggc ccc aac tgc gag cgg ccc tac gtg ccc tgc agc ccc	672
Thr His Thr Gly Pro Asn Cys Glu Arg Pro Tyr Val Pro Cys Ser Pro	
210 215 220	
tcg ccc tgc cag aac ggg ggc acc tgc cgc ccc acg ggc gac gtc acc	720
Ser Pro Cys Gln Asn Gly Gly Thr Cys Arg Pro Thr Gly Asp Val Thr	
225 230 235 240	
cac gag tgt gcc tgc ctg cca ggc ttc acc ggc cag aac tgt gag gaa	768
His Glu Cys Ala Cys Leu Pro Gly Phe Thr Gly Gln Asn Cys Glu Glu	
245 250 255	
aat atc gac gat tgt cca gga aac aac tgc aag aac ggg ggt gcc tgt	816
Asn Ile Asp Asp Cys Pro Gly Asn Asn Cys Lys Asn Gly Gly Ala Cys	
260 265 270	
gtg gac ggc gtg aac acc tac aac tgc ccg tgc ccg cca gag tgg aca	864
Val Asp Gly Val Asn Thr Tyr Asn Cys Pro Cys Pro Pro Glu Trp Thr	
275 280 285	

- 79 -

ggt cag tac tgt acc gag gat gtg gac gag tgc cag ctg atg cca aat	912
Gly Gln Tyr Cys Thr Glu Asp Val Asp Glu Cys Gln Leu Met Pro Asn	
290 295 300	
gcc tgc cag aac ggc ggg acc tgc cac aac acc cac ggt ggc tac aac	960
Ala Cys Gln Asn Gly Gly Thr Cys His Asn Thr His Gly Gly Tyr Asn	
305 310 315 320	
tgc gtg tgt gtc aac ggc tgg act ggt gag gac tgc agc gag aac att	1008
Cys Val Cys Val Asn Gly Trp Thr Gly Glu Asp Cys Ser Glu Asn Ile	
325 330 335	
gat gac tgt gcc agc gcc gcc tgc ttc cac ggc gcc acc tgc cat gac	1056
Asp Asp Cys Ala Ser Ala Ala Cys Phe His Gly Ala Thr Cys His Asp	
340 345 350	
cgt gtg gcc tcc ttt tac tgc gag tgt ccc cat ggc cgc aca ggt ctg	1104
Arg Val Ala Ser Phe Tyr Cys Glu Cys Pro His Gly Arg Thr Gly Leu	
355 360 365	
ctg tgc cac ctc aac gac gca tgc atc agc aac ccc tgt aac gag ggc	1152
Leu Cys His Leu Asn Asp Ala Cys Ile Ser Asn Pro Cys Asn Glu Gly	
370 375 380	
tcc aac tgc gac acc aac cct gtc aat ggc aag gcc atc tgc acc tgc	1200
Ser Asn Cys Asp Thr Asn Pro Val Asn Gly Lys Ala Ile Cys Thr Cys	
385 390 395 400	
ccc tcg ggg tac acg ggc ccg gcc tgc agc cag gac gtg gat gag tgc	1248
Pro Ser Gly Tyr Thr Gly Pro Ala Cys Ser Gln Asp Val Asp Glu Cys	
405 410 415	
tcg ctg ggt gcc aac ccc tgc gag cat gcg ggc aag tgc atc aac acg	1296
Ser Leu Gly Ala Asn Pro Cys Glu His Ala Gly Lys Cys Ile Asn Thr	
420 425 430	
ctg ggc tcc ttc gag tgc cag tgt ctg cag ggc tac acg ggc ccc cga	1344
Leu Gly Ser Phe Glu Cys Gln Cys Leu Gln Gly Tyr Thr Gly Pro Arg	
435 440 445	
tgc gag atc gac gtc aac gag tgc gtc tcg aac ccg tgc cag aac gac	1392
Cys Glu Ile Asp Val Asn Glu Cys Val Ser Asn Pro Cys Gln Asn Asp	
450 455 460	
gcc acc tgc ctg gac cag att ggg gag ttc cag tgc atg tgc atg ccc	1440
Ala Thr Cys Leu Asp Gln Ile Gly Glu Phe Gln Cys Met Cys Met Pro	
465 470 475 480	
ggc tac gag ggt gtg cac tgc gag gtc aac aca gac gag tgt gcc agc	1488
Gly Tyr Glu Gly Val His Cys Glu Val Asn Thr Asp Glu Cys Ala Ser	
485 490 495	

- 80 -

agc ccc tgc ctg cac aat ggc cgc tgc ctg gac aag atc aat gag ttc	1536
Ser Pro Cys Leu His Asn Gly Arg Cys Leu Asp Lys Ile Asn Glu Phe	
500 505 510	
cag tgc gag tgc ccc acg ggc ttc act ggg cat ctg tgc cag tac gat	1584
Gln Cys Glu Cys Pro Thr Gly Phe Thr Gly His Leu Cys Gln Tyr Asp	
515 520 525	
gtg gac gag tgt gcc agc acc ccc tgc aag aat ggt gcc aag tgc ctg	1632
Val Asp Glu Cys Ala Ser Thr Pro Cys Lys Asn Gly Ala Lys Cys Leu	
530 535 540	
gac gga ccc aac act tac acc tgt gtg tgc acg gaa ggg tac acg ggg	1680
Asp Gly Pro Asn Thr Tyr Thr Cys Val Cys Thr Glu Gly Tyr Thr Gly	
545 550 555 560	
acg cac tgc gag gtg gac atc gat gag tgc gac ccc gac ccc tgc cac	1728
Thr His Cys Glu Val Asp Ile Asp Glu Cys Asp Pro Asp Pro Cys His	
565 570 575	
tac ggc tcc tgc aag gac ggc gtc gcc acc ttc acc tgc ctc tgc cgc	1776
Tyr Gly Ser Cys Lys Asp Gly Val Ala Thr Phe Thr Cys Leu Cys Arg	
580 585 590	
cca ggc tac acg ggc cac cac tgc gag acc aac atc aac gag tgc tcc	1824
Pro Gly Tyr Thr Gly His His Cys Glu Thr Asn Ile Asn Glu Cys Ser	
595 600 605	
agc cag ccc tgc cgc cta cgg ggc acc tgc cag gac ccg gac aac gcc	1872
Ser Gln Pro Cys Arg Leu Arg Gly Thr Cys Gln Asp Pro Asp Asn Ala	
610 615 620	
tac ctc tgc ttc tgc ctg aag ggg acc aca gga ccc aac tgc gag atc	1920
Tyr Leu Cys Phe Cys Leu Lys Gly Thr Thr Gly Pro Asn Cys Glu Ile	
625 630 635 640	
aac ctg gat gac tgt gcc agc agc ccc tgc gac tcg ggc acc tgt ctg	1968
Asn Leu Asp Asp Cys Ala Ser Ser Pro Cys Asp Ser Gly Thr Cys Leu	
645 650 655	
gac aag atc gat ggc tac gag tgt gcc tgt gag ccg ggc tac aca ggg	2016
Asp Lys Ile Asp Gly Tyr Glu Cys Ala Cys Glu Pro Gly Tyr Thr Gly	
660 665 670	
agc atg tgt aac agc aac atc gat gag tgt gcg ggc aac ccc tgc cac	2064
Ser Met Cys Asn Ser Asn Ile Asp Glu Cys Ala Gly Asn Pro Cys His	
675 680 685	
aac ggg ggc acc tgc gag gac ggc atc aat ggc ttc acc tgc cgc tgc	2112
Asn Gly Gly Thr Cys Glu Asp Gly Ile Asn Gly Phe Thr Cys Arg Cys	
690 695 700	

- 81 -

ccc gag ggc tac cac gac ccc acc tgc ctg tct gag gtc aat gag tgc	2160
Pro Glu Gly Tyr His Asp Pro Thr Cys Leu Ser Glu Val Asn Glu Cys	
705 710 715 720	
aac agc aac ccc tgc gtc cac ggg gcc tgc cgg gac agc ctc aac ggg	2208
Asn Ser Asn Pro Cys Val His Gly Ala Cys Arg Asp Ser Leu Asn Gly	
725 730 735	
tac aag tgc gac tgt gac cct ggg tgg agt ggg acc aac tgt gac atc	2256
Tyr Lys Cys Asp Cys Asp Pro Gly Trp Ser Gly Thr Asn Cys Asp Ile	
740 745 750	
aac aac aac gag tgt gaa tcc aac cct tgt gtc aac ggc ggc acc tgc	2304
Asn Asn Asn Glu Cys Glu Ser Asn Pro Cys Val Asn Gly Gly Thr Cys	
755 760 765	
aaa gac atg acc agt ggc atc gtg tgc acc tgc cgg gag ggc ttc agc	2352
Lys Asp Met Thr Ser Gly Ile Val Cys Thr Cys Arg Glu Gly Phe Ser	
770 775 780	
ggt ccc aac tgc cag acc aac atc aac gag tgt gcg tcc aac cca tgt	2400
Gly Pro Asn Cys Gln Thr Asn Ile Asn Glu Cys Ala Ser Asn Pro Cys	
785 790 795 800	
ctg aac aag ggc acg tgt att gac gac gtt gcc ggg tac aag tgc aac	2448
Leu Asn Lys Gly Thr Cys Ile Asp Asp Val Ala Gly Tyr Lys Cys Asn	
805 810 815	
tgc ctg ctg ccc tac aca ggt gcc acg tgt gag gtg gtg ctg gcc ccg	2496
Cys Leu Leu Pro Tyr Thr Gly Ala Thr Cys Glu Val Val Leu Ala Pro	
820 825 830	
tgt gcc ccc agc ccc tgc aga aac ggc ggg gag tgc agg caa tcc gag	2544
Cys Ala Pro Ser Pro Cys Arg Asn Gly Gly Glu Cys Arg Gln Ser Glu	
835 840 845	
gac tat gag agc ttc tcc tgt gtc tgc ccc acg gct ggg gcc aaa ggg	2592
Asp Tyr Glu Ser Phe Ser Cys Val Cys Pro Thr Ala Gly Ala Lys Gly	
850 855 860	
cag acc tgt gag gtc gac atc aac gag tgc gtt ctg agc ccg tgc cgg	2640
Gln Thr Cys Glu Val Asp Ile Asn Glu Cys Val Leu Ser Pro Cys Arg	
865 870 875 880	
cac ggc gca tcc tgc cag aac acc cac ggc gss tac cgc tgc cac tgc	2688
His Gly Ala Ser Cys Gln Asn Thr His Gly Xaa Tyr Arg Cys His Cys	
885 890 895	
cag gcc ggc tac agt ggg cgc aac tgc gag acc gac atc gac gac tgc	2736
Gln Ala Gly Tyr Ser Gly Arg Asn Cys Glu Thr Asp Ile Asp Asp Cys	
900 905 910	

- 82 -

cgg ccc aac ccg tgt cac aac ggg ggc tcc tgc aca gac ggc atc aac	2784
Arg Pro Asn Pro Cys His Asn Gly Gly Ser Cys Thr Asp Gly Ile Asn	
915 920 925	
acg gcc ttc tgc gac tgc ctg ccc ggc ttc cgg ggc act ttc tgt gag	2832
Thr Ala Phe Cys Asp Cys Leu Pro Gly Phe Arg Gly Thr Phe Cys Glu	
930 935 940	
gag gac atc aac gag tgt gcc agt gac ccc tgc cgc aac ggg gcc aac	2880
Glu Asp Ile Asn Glu Cys Ala Ser Asp Pro Cys Arg Asn Gly Ala Asn	
945 950 955 960	
tgc acg gac tgc gtg gac agc tac acg tgc acc tgc ccc gca ggc ttc	2928
Cys Thr Asp Cys Val Asp Ser Tyr Thr Cys Thr Cys Pro Ala Gly Phe	
965 970 975	
agc ggg atc cac tgt gag aac aac acg cct gac tgc aca gag agc tcc	2976
Ser Gly Ile His Cys Glu Asn Asn Thr Pro Asp Cys Thr Glu Ser Ser	
980 985 990	
tgc ttc aac ggt ggc acc tgc gtg gac ggc atc aac tcg ttc acc tgc	3024
Cys Phe Asn Gly Gly Thr Cys Val Asp Gly Ile Asn Ser Phe Thr Cys	
995 1000 1005	
ctg tgt cca ccc ggc ttc acg ggc agc tac tgc cag cac gta gtc aat	3072
Leu Cys Pro Pro Gly Phe Thr Gly Ser Tyr Cys Gln His Val Val Asn	
1010 1015 1020	
gag tgc gac tca cga ccc tgc ctg cta ggc ggc acc tgt cag gac ggt	3120
Glu Cys Asp Ser Arg Pro Cys Leu Leu Gly Gly Thr Cys Gln Asp Gly	
1025 1030 1035 1040	
cgc ggt ctc cac agg tgc acc tgc ccc cag ggc tac act ggc ccc aac	3168
Arg Gly Leu His Arg Cys Thr Cys Pro Gln Gly Tyr Thr Gly Pro Asn	
1045 1050 1055	
tgc cag aac ctt gtg cac tgg tgt gac tcc tcg ccc tgc aag aac ggc	3216
Cys Gln Asn Leu Val His Trp Cys Asp Ser Ser Pro Cys Lys Asn Gly	
1060 1065 1070	
ggc aaa tgc tgg cag acc cac acc cag tac cgc tgc gag tgc ccc agc	3264
Gly Lys Cys Trp Gln Thr His Thr Gln Tyr Arg Cys Glu Cys Pro Ser	
1075 1080 1085	
ggc tgg acc ggc ctt tac tgc gac gtg ccc agc gtg tcc tgt gag gtg	3312
Gly Trp Thr Gly Leu Tyr Cys Asp Val Pro Ser Val Ser Cys Glu Val	
1090 1095 1100	
gct gcg cag cga caa ggt gtt gac gtt gcc cgc ctg tgc cag cat gga	3360
Ala Ala Gln Arg Gln Gly Val Asp Val Ala Arg Leu Cys Gln His Gly	
1105 1110 1115 1120	

- 83 -

```

ggg ctc tgt gtg gac gcg ggc aac acg cac cac tgc cgc tgc cag gcg 3408
Gly Leu Cys Val Asp Ala Gly Asn Thr His His Cys Arg Cys Gln Ala
      1125              1130              1135

ggc tac aca ggc agc tac tgt gag gac ctg gtg gac gag tgc tca ccc 3456
Gly Tyr Thr Gly Ser Tyr Cys Glu Asp Leu Val Asp Glu Cys Ser Pro
      1140              1145              1150

agc ccc tgc cag aac ggg gcc acc tgc acg gac tac ctg ggc ggc tac 3504
Ser Pro Cys Gln Asn Gly Ala Thr Cys Thr Asp Tyr Leu Gly Gly Tyr
      1155              1160              1165

tcc tgc aag tgc gtg gcc ggc tac cac ggg gtg aac tgc tct gag gag 3552
Ser Cys Lys Cys Val Ala Gly Tyr His Gly Val Asn Cys Ser Glu Glu
      1170              1175              1180

atc gac gag tgc ctc tcc cac ccc tgc cag aac ggg ggc acc tgc ctc 3600
Ile Asp Glu Cys Leu Ser His Pro Cys Gln Asn Gly Gly Thr Cys Leu
1185              1190              1195              1200

gac ctc ccc aac acc tac aag tgc tcc tgc cca cgg ggc act cag ggt 3648
Asp Leu Pro Asn Thr Tyr Lys Cys Ser Cys Pro Arg Gly Thr Gln Gly
      1205              1210              1215

gtg cac tgt gag atc aac gtg gac gac tgc aat ccc ccc gtt gac ccc 3696
Val His Cys Glu Ile Asn Val Asp Asp Cys Asn Pro Pro Val Asp Pro
      1220              1225              1230

gtg tcc cgg agc ccc aag tgc ttt aac aac ggc acc tgc gtg gac cag 3744
Val Ser Arg Ser Pro Lys Cys Phe Asn Asn Gly Thr Cys Val Asp Gln
      1235              1240              1245

gtg ggc ggc tac agc tgc acc tgc ccg ccg ggc ttc gtg ggt gag cgc 3792
Val Gly Gly Tyr Ser Cys Thr Cys Pro Pro Gly Phe Val Gly Glu Arg
      1250              1255              1260

tgt gag ggg gat gtc aac gag tgc ctg tcc aat ccc tgc gac gcc cgt 3840
Cys Glu Gly Asp Val Asn Glu Cys Leu Ser Asn Pro Cys Asp Ala Arg
1265              1270              1275              1280

ggc acc cag aac tgc gtg cag cgc gtc aat gac ttc cac tgc gag tgc 3888
Gly Thr Gln Asn Cys Val Gln Arg Val Asn Asp Phe His Cys Glu Cys
      1285              1290              1295

cgt gct ggt cac acc ggg cgc cgc tgc gag tcc gtc atc aat ggc tgc 3936
Arg Ala Gly His Thr Gly Arg Arg Cys Glu Ser Val Ile Asn Gly Cys
      1300              1305              1310

aaa ggc aag ccc tgc aag aat ggg ggc acc tgc gcc gtg gcc tcc aac 3984
Lys Gly Lys Pro Cys Lys Asn Gly Gly Thr Cys Ala Val Ala Ser Asn
      1315              1320              1325

```

- 84 -

acc gcc cgc ggg ttc atc tgc aag tgc cct gcg ggc ttc gag ggc gcc 4032
 Thr Ala Arg Gly Phe Ile Cys Lys Cys Pro Ala Gly Phe Glu Gly Ala
 1330 1335 1340

acg tgt gag aat gac gct cgt acc tgc ggc agc ctg cgc tgc ctc aac 4080
 Thr Cys Glu Asn Asp Ala Arg Thr Cys Gly Ser Leu Arg Cys Leu Asn
 1345 1350 1355 1360

ggc ggc aca tgc atc tcc ggc ccg cgc agc ccc acc tgc ctg tgc ctg 4128
 Gly Gly Thr Cys Ile Ser Gly Pro Arg Ser Pro Thr Cys Leu Cys Leu
 1365 1370 1375

ggc ccc ttc acg ggc ccc gaa tgc cag ttc ccg gcc agc agc ccc tgc 4176
 Gly Pro Phe Thr Gly Pro Glu Cys Gln Phe Pro Ala Ser Ser Pro Cys
 1380 1385 1390

ctg ggc ggc aac ccc tgc tac aac cag ggg acc tgt gag ccc aca tcc 4224
 Leu Gly Gly Asn Pro Cys Tyr Asn Gln Gly Thr Cys Glu Pro Thr Ser
 1395 1400 1405

gag agc ccc ttc tac cgt tgc ctg tgc ccc gcc aaa ttc aac ggc ctc 4272
 Glu Ser Pro Phe Tyr Arg Cys Leu Cys Pro Ala Lys Phe Asn Gly Leu
 1410 1415 1420

ttg tgc cac atc ctg gac tac agc ttc ggg ggt ggg gcc ggg cgc gac 4320
 Leu Cys His Ile Leu Asp Tyr Ser Phe Gly Gly Gly Ala Gly Arg Asp
 1425 1430 1435 1440

atc ccc ccg ccg ctg atc gag gag gcg tgc gag ctg ccc gag tgc cag 4368
 Ile Pro Pro Pro Leu Ile Glu Glu Ala Cys Glu Leu Pro Glu Cys Gln
 1445 1450 1455

gag gac gcg ggc aac aag gtc tgc agc ctg cag tgc aac aac cac gcg 4416
 Glu Asp Ala Gly Asn Lys Val Cys Ser Leu Gln Cys Asn Asn His Ala
 1460 1465 1470

tgc ggc tgg gac ggc ggt gac tgc tcc ctc aac ttc aat gac ccc tgg 4464
 Cys Gly Trp Asp Gly Gly Asp Cys Ser Leu Asn Phe Asn Asp Pro Trp
 1475 1480 1485

aag aac tgc acg cag tct ctg cag tgc tgg aag tac ttc agt gac ggc 4512
 Lys Asn Cys Thr Gln Ser Leu Gln Cys Trp Lys Tyr Phe Ser Asp Gly
 1490 1495 1500

cac tgt gac agc cag tgc aac tca gcc ggc tgc ctc ttc gac ggc ttt 4560
 His Cys Asp Ser Gln Cys Asn Ser Ala Gly Cys Leu Phe Asp Gly Phe
 1505 1510 1515 1520

gac tgc cag cgt gcg gaa ggc cag tgc aac ccc ctg tac gac cag tac 4608
 Asp Cys Gln Arg Ala Glu Gly Gln Cys Asn Pro Leu Tyr Asp Gln Tyr
 1525 1530 1535

- 85 -

tgc aag gac cac ttc agc gac ggg cac tgc gac cag ggc tgc aac agc	4656
Cys Lys Asp His Phe Ser Asp Gly His Cys Asp Gln Gly Cys Asn Ser	
1540 1545 1550	
gcg gag tgc gag tgg gac ggg ctg gac tgt gcg gag cat gta ccc gag	4704
Ala Glu Cys Glu Trp Asp Gly Leu Asp Cys Ala Glu His Val Pro Glu	
1555 1560 1565	
agg ctg gcg gcc ggc acg ctg gtg gtg gtg ctg atg ccg ccg gag	4752
Arg Leu Ala Ala Gly Thr Leu Val Val Val Val Leu Met Pro Pro Glu	
1570 1575 1580	
cag ctg cgc aac agc tcc ttc cac ttc ctg cgg gag ctc agc cgc gtg	4800
Gln Leu Arg Asn Ser Ser Phe His Phe Leu Arg Glu Leu Ser Arg Val	
1585 1590 1595 1600	
ctg cac acc aac gtg gtc ttc aag cgt gac gca cac ggc cag cag atg	4848
Leu His Thr Asn Val Val Phe Lys Arg Asp Ala His Gly Gln Gln Met	
1605 1610 1615	
atc ttc ccc tac tac ggc cgc gag gag gag ctg cgc aag cac ccc atc	4896
Ile Phe Pro Tyr Tyr Gly Arg Glu Glu Glu Leu Arg Lys His Pro Ile	
1620 1625 1630	
aag cgt gcc gcc gag ggc tgg gcc gca cct gac gcc ctg ctg ggc cag	4944
Lys Arg Ala Ala Glu Gly Trp Ala Ala Pro Asp Ala Leu Leu Gly Gln	
1635 1640 1645	
gtg aag gcc tcg ctg ctc cct ggt ggc agc gag ggt ggg cgg cgg cgg	4992
Val Lys Ala Ser Leu Leu Pro Gly Gly Ser Glu Gly Gly Arg Arg Arg	
1650 1655 1660	
agg gag ctg gac ccc atg gac gtc cgc ggc tcc atc gtc tac ctg gag	5040
Arg Glu Leu Asp Pro Met Asp Val Arg Gly Ser Ile Val Tyr Leu Glu	
1665 1670 1675 1680	
att gac aac cgg cag tgt gtg cag gcc tcc tcg cag tgc ttc cag agt	5088
Ile Asp Asn Arg Gln Cys Val Gln Ala Ser Ser Gln Cys Phe Gln Ser	
1685 1690 1695	
gcc acc gac gtg gcc gca ttc ctg gga gcg ctc gcc tcg ctg ggc agc	5136
Ala Thr Asp Val Ala Ala Phe Leu Gly Ala Leu Ala Ser Leu Gly Ser	
1700 1705 1710	
ctc aac atc ccc tac aag atc gag gcc gtg cag agt gag acc gtg gag	5184
Leu Asn Ile Pro Tyr Lys Ile Glu Ala Val Gln Ser Glu Thr Val Glu	
1715 1720 1725	
ccg ccc ccg ccg gcg cag ctg cac ttc atg tac gtg gcg gcg gcc gcc	5232
Pro Pro Pro Pro Ala Gln Leu His Phe Met Tyr Val Ala Ala Ala Ala	
1730 1735 1740	

- 86 -

ttt gtg ctt ctg ttc ttc gtg ggc tgc ggg gtg ctg ctg tcc cgc aag	5280
Phe Val Leu Leu Phe Phe Val Gly Cys Gly Val Leu Leu Ser Arg Lys	
1745 1750 1755 1760	
cgc cgg cgg cag cat ggc cag ctc tgg ttc cct gag ggc ttc aaa gtg	5328
Arg Arg Arg Gln His Gly Gln Leu Trp Phe Pro Glu Gly Phe Lys Val	
1765 1770 1775	
tct gag gcc agc aag aag aag cgg cgg gag ccc ctc ggc gag gac tcc	5376
Ser Glu Ala Ser Lys Lys Lys Arg Arg Glu Pro Leu Gly Glu Asp Ser	
1780 1785 1790	
gtg ggc ctc aag ccc ctg aag aac gct tca gac ggt gcc ctc atg gac	5424
Val Gly Leu Lys Pro Leu Lys Asn Ala Ser Asp Gly Ala Leu Met Asp	
1795 1800 1805	
gac aac cag aat gag tgg ggg gac gag gac ctg gag acc aag aag ttc	5472
Asp Asn Gln Asn Glu Trp Gly Asp Glu Asp Leu Glu Thr Lys Lys Phe	
1810 1815 1820	
cgg ttc gag gag ccc gtg gtt ctg cct gac ctg gac gac cag aca gac	5520
Arg Phe Glu Glu Pro Val Val Leu Pro Asp Leu Asp Asp Gln Thr Asp	
1825 1830 1835 1840	
cac cgg cag tgg act cag cag cac ctg gat gcc gct gac ctg cgc atg	5568
His Arg Gln Trp Thr Gln Gln His Leu Asp Ala Ala Asp Leu Arg Met	
1845 1850 1855	
tct gcc atg gcc ccc aca ccg ccc cag ggt gag gtt gac gcc gac tgc	5616
Ser Ala Met Ala Pro Thr Pro Pro Gln Gly Glu Val Asp Ala Asp Cys	
1860 1865 1870	
atg gac gtc aat gtc cgc ggg cct gat ggc ttc acc ccg ctc atg atc	5664
Met Asp Val Asn Val Arg Gly Pro Asp Gly Phe Thr Pro Leu Met Ile	
1875 1880 1885	
gcc tcc tgc agc ggg ggc ggc ctg gag acg ggc aac agc gag gaa gag	5712
Ala Ser Cys Ser Gly Gly Gly Leu Glu Thr Gly Asn Ser Glu Glu Glu	
1890 1895 1900	
gag gac gcg ccg gcc gtc atc tcc gac ttc atc tac cag ggc gcc agc	5760
Glu Asp Ala Pro Ala Val Ile Ser Asp Phe Ile Tyr Gln Gly Ala Ser	
1905 1910 1915 1920	
ctg cac aac cag aca gac cgc acg ggc gag acc gcc ttg cac ctg gcc	5808
Leu His Asn Gln Thr Asp Arg Thr Gly Glu Thr Ala Leu His Leu Ala	
1925 1930 1935	
gcc cgc tac tca cgc tct gat gcc gcc aag cgc ctg ctg gag gcc agc	5856
Ala Arg Tyr Ser Arg Ser Asp Ala Ala Lys Arg Leu Leu Glu Ala Ser	
1940 1945 1950	

- 87 -

gca gat gcc aac atc cag gac aac atg ggc cgc acc ccg ctg cat gcg	5904
Ala Asp Ala Asn Ile Gln Asp Asn Met Gly Arg Thr Pro Leu His Ala	
1955 1960 1965	
gct gtg tct gcc gac gca caa ggt gtc ttc cag atc ctg atc cgg aac	5952
Ala Val Ser Ala Asp Ala Gln Gly Val Phe Gln Ile Leu Ile Arg Asn	
1970 1975 1980	
cga gcc aca gac ctg gat gcc cgc atg cat gat ggc acg acg cca ctg	6000
Arg Ala Thr Asp Leu Asp Ala Arg Met His Asp Gly Thr Thr Pro Leu	
1985 1990 1995 2000	
atc ctg gct gcc cgc ctg gcc gtg gag ggc atg ctg gag gac ctc atc	6048
Ile Leu Ala Ala Arg Leu Ala Val Glu Gly Met Leu Glu Asp Leu Ile	
2005 2010 2015	
aac tca cac gcc gac gtc aac gcc gta gat gac ctg ggc aag tcc gcc	6096
Asn Ser His Ala Asp Val Asn Ala Val Asp Asp Leu Gly Lys Ser Ala	
2020 2025 2030	
ctg cac tgg gcc gcc gcc gtg aac aat gtg gat gcc gca gtt gtg ctc	6144
Leu His Trp Ala Ala Ala Val Asn Asn Val Asp Ala Ala Val Val Leu	
2035 2040 2045	
ctg aag aac ggg gct aac aaa gat atg cag aac aac agg gag gag aca	6192
Leu Lys Asn Gly Ala Asn Lys Asp Met Gln Asn Asn Arg Glu Glu Thr	
2050 2055 2060	
ccc ctg ttt ctg gcc gcc cgg gag ggc agc tac gag acc gcc aag gtg	6240
Pro Leu Phe Leu Ala Ala Arg Glu Gly Ser Tyr Glu Thr Ala Lys Val	
2065 2070 2075 2080	
ctg ctg gac cac ttt gcc aac cgg gac atc acg gat cat atg gac cgc	6288
Leu Leu Asp His Phe Ala Asn Arg Asp Ile Thr Asp His Met Asp Arg	
2085 2090 2095	
ctg ccg cgc gac atc gca cag gag cgc atg cat cac gac atc gtg agg	6336
Leu Pro Arg Asp Ile Ala Gln Glu Arg Met His His Asp Ile Val Arg	
2100 2105 2110	
ctg ctg gac gag tac aac ctg gtg cgc agc ccg cag ctg cac gga gcc	6384
Leu Leu Asp Glu Tyr Asn Leu Val Arg Ser Pro Gln Leu His Gly Ala	
2115 2120 2125	
ccg ctg ggg ggc acg ccc acc ctg tgc ccc ccg ctc tgc tgc ccc aac	6432
Pro Leu Gly Gly Thr Pro Thr Leu Ser Pro Pro Leu Cys Ser Pro Asn	
2130 2135 2140	
ggc tac ctg ggc agc ctc aag ccc ggc gtg cag ggc aag aag gtc cgc	6480
Gly Tyr Leu Gly Ser Leu Lys Pro Gly Val Gln Gly Lys Lys Val Arg	
2145 2150 2155 2160	

- 88 -

aag ccc agc agc aaa ggc ctg gcc tgt gga agc aag gag gcc aag gac	6528
Lys Pro Ser Ser Lys Gly Leu Ala Cys Gly Ser Lys Glu Ala Lys Asp	
2165 2170 2175	
ctc aag gca cgg agg aag aag tcc cag gat ggc aag ggc tgc ctg ctg	6576
Leu Lys Ala Arg Arg Lys Lys Ser Gln Asp Gly Lys Gly Cys Leu Leu	
2180 2185 2190	
gac agc tcc ggc atg ctc tcg ccc gtg gac tcc ctg gag tca ccc cat	6624
Asp Ser Ser Gly Met Leu Ser Pro Val Asp Ser Leu Glu Ser Pro His	
2195 2200 2205	
ggc tac ctg tca gac gtg gcc tcg ccg cca ctg ctg ccc tcc ccg ttc	6672
Gly Tyr Leu Ser Asp Val Ala Ser Pro Pro Leu Leu Pro Ser Pro Phe	
2210 2215 2220	
cag cag tct ccg tcc gtg ccc ctc aac cac ctg cct ggg atg ccc gac	6720
Gln Gln Ser Pro Ser Val Pro Leu Asn His Leu Pro Gly Met Pro Asp	
2225 2230 2235 2240	
acc cac ctg ggc atc ggg cac ctg aac gtg gcg gcc aag ccc gag atg	6768
Thr His Leu Gly Ile Gly His Leu Asn Val Ala Ala Lys Pro Glu Met	
2245 2250 2255	
gcg gcg ctg ggt ggg ggc ggc cgg ctg gcc ttt gag act ggc cca cct	6816
Ala Ala Leu Gly Gly Gly Arg Leu Ala Phe Glu Thr Gly Pro Pro	
2260 2265 2270	
cgt ctc tcc cac ctg cct gtg gcc tct ggc acc agc acc gtc ctg ggc	6864
Arg Leu Ser His Leu Pro Val Ala Ser Gly Thr Ser Thr Val Leu Gly	
2275 2280 2285	
tcc agc agc gga ggg gcc ctg aat ttc act gtg ggc ggg tcc acc agt	6912
Ser Ser Gly Gly Ala Leu Asn Phe Thr Val Gly Gly Ser Thr Ser	
2290 2295 2300	
ttg aat ggt caa tgc gag tgg ctg tcc cgg ctg cag agc ggc atg gtg	6960
Leu Asn Gly Gln Cys Glu Trp Leu Ser Arg Leu Gln Ser Gly Met Val	
2305 2310 2315 2320	
ccg aac caa tac aac cct ctg cgg ggg agt gtg gca cca ggc ccc ctg	7008
Pro Asn Gln Tyr Asn Pro Leu Arg Gly Ser Val Ala Pro Gly Pro Leu	
2325 2330 2335	
agc aca cag gcc ccc tcc ctg cag cat ggc atg gta ggc ccg ctg cac	7056
Ser Thr Gln Ala Pro Ser Leu Gln His Gly Met Val Gly Pro Leu His	
2340 2345 2350	
agt agc ctt gct gcc agc gcc ctg tcc cag atg atg agc tac cag ggc	7104
Ser Ser Leu Ala Ala Ser Ala Leu Ser Gln Met Met Ser Tyr Gln Gly	
2355 2360 2365	

- 89 -

ctg ccc agc acc cgg ctg gcc acc cag cct cac ctg gtg cag acc cag 7152
 Leu Pro Ser Thr Arg Leu Ala Thr Gln Pro His Leu Val Gln Thr Gln
 2370 2375 2380

cag gtg cag cca caa aac tta cag atg cag cag cag aac ctg cag cca 7200
 Gln Val Gln Pro Gln Asn Leu Gln Met Gln Gln Gln Asn Leu Gln Pro
 2385 2390 2395 2400

gca aac atc cag cag cag caa agc ctg cag ccg cca cca cca cca cca 7248
 Ala Asn Ile Gln Gln Gln Gln Ser Leu Gln Pro Pro Pro Pro Pro Pro
 2405 2410 2415

cag ccg cac ctt ggc gtg agc tca gca gcc agc ggc cac ctg ggc cgg 7296
 Gln Pro His Leu Gly Val Ser Ser Ala Ala Ser Gly His Leu Gly Arg
 2420 2425 2430

agc ttc ctg agt gga gag ccg agc cag gca gac gtg cag cca ctg ggc 7344
 Ser Phe Leu Ser Gly Glu Pro Ser Gln Ala Asp Val Gln Pro Leu Gly
 2435 2440 2445

ccc agc agc ctg gcg gtg cac act att ctg ccc cag gag agc ccc gcc 7392
 Pro Ser Ser Leu Ala Val His Thr Ile Leu Pro Gln Glu Ser Pro Ala
 2450 2455 2460

ctg ccc acg tcg ctg cca tcc tcg ctg gtc cca ccc gtg acc gca gcc 7440
 Leu Pro Thr Ser Leu Pro Ser Ser Leu Val Pro Pro Val Thr Ala Ala
 2465 2470 2475 2480

cag ttc ctg acg ccc ccc tcg cag cac agc tac tcc tcg cct gtg gac 7488
 Gln Phe Leu Thr Pro Pro Ser Gln His Ser Tyr Ser Ser Pro Val Asp
 2485 2490 2495

aac acc ccc agc cac cag cta cag gtg cct gag cac ccc ttc ctg acc 7536
 Asn Thr Pro Ser His Gln Leu Gln Val Pro Glu His Pro Phe Leu Thr
 2500 2505 2510

cct tcg ccg gag tcg ccc gac caa tgg tcg tcc tcg tcg ccg cac tct 7584
 Pro Ser Pro Glu Ser Pro Asp Gln Trp Ser Ser Ser Ser Pro His Ser
 2515 2520 2525

aat gtg tct gac tgg tct gag ggc gtg tcg tcg ccc ccg acc tcc atg 7632
 Asn Val Ser Asp Trp Ser Glu Gly Val Ser Ser Pro Pro Thr Ser Met
 2530 2535 2540

cag tcc cag atc gcg cgc atc ccg gag gcg ttc aag taa 7671
 Gln Ser Gln Ile Ala Arg Ile Pro Glu Ala Phe Lys
 2545 2550 2555

<210> 32

<211> 2556

- 90 -

<212> PRT

<213> Homo Sapiens

<220>

<221> VARIANT

<222> 891

<223> Xaa is Gly or Ala

<300>

<308> Genbank AF308602

<309> 2000-11-22

<400> 32

```

Met Pro Pro Leu Leu Ala Pro Leu Leu Cys Leu Ala Leu Leu Pro Ala
 1           5           10           15
Leu Ala Ala Arg Gly Pro Arg Cys Ser Gln Pro Gly Glu Thr Cys Leu
           20           25           30
Asn Gly Gly Lys Cys Glu Ala Ala Asn Gly Thr Glu Ala Cys Val Cys
           35           40           45
Gly Gly Ala Phe Val Gly Pro Arg Cys Gln Asp Pro Asn Pro Cys Leu
           50           55           60
Ser Thr Pro Cys Lys Asn Ala Gly Thr Cys His Val Val Asp Arg Arg
           65           70           75           80
Gly Val Ala Asp Tyr Ala Cys Ser Cys Ala Leu Gly Phe Ser Gly Pro
           85           90           95
Leu Cys Leu Thr Pro Leu Asp Asn Ala Cys Leu Thr Asn Pro Cys Arg
           100          105          110
Asn Gly Gly Thr Cys Asp Leu Leu Thr Leu Thr Glu Tyr Lys Cys Arg
           115          120          125
Cys Pro Pro Gly Trp Ser Gly Lys Ser Cys Gln Gln Ala Asp Pro Cys
           130          135          140
Ala Ser Asn Pro Cys Ala Asn Gly Gly Gln Cys Leu Pro Phe Glu Ala
           145          150          155          160
Ser Tyr Ile Cys His Cys Pro Pro Ser Phe His Gly Pro Thr Cys Arg
           165          170          175
Gln Asp Val Asn Glu Cys Gly Gln Lys Pro Arg Leu Cys Arg His Gly
           180          185          190
Gly Thr Cys His Asn Glu Val Gly Ser Tyr Arg Cys Val Cys Arg Ala
           195          200          205
Thr His Thr Gly Pro Asn Cys Glu Arg Pro Tyr Val Pro Cys Ser Pro
           210          215          220
Ser Pro Cys Gln Asn Gly Gly Thr Cys Arg Pro Thr Gly Asp Val Thr
           225          230          235          240
His Glu Cys Ala Cys Leu Pro Gly Phe Thr Gly Gln Asn Cys Glu Glu
           245          250          255
Asn Ile Asp Asp Cys Pro Gly Asn Asn Cys Lys Asn Gly Gly Ala Cys
           260          265          270
Val Asp Gly Val Asn Thr Tyr Asn Cys Pro Cys Pro Pro Glu Trp Thr
           275          280          285
Gly Gln Tyr Cys Thr Glu Asp Val Asp Glu Cys Gln Leu Met Pro Asn
           290          295          300
Ala Cys Gln Asn Gly Gly Thr Cys His Asn Thr His Gly Gly Tyr Asn

```


- 91 -

```

305          310          315          320
Cys Val Cys Val Asn Gly Trp Thr Gly Glu Asp Cys Ser Glu Asn Ile
          325          330          335
Asp Asp Cys Ala Ser Ala Ala Cys Phe His Gly Ala Thr Cys His Asp
          340          345          350
Arg Val Ala Ser Phe Tyr Cys Glu Cys Pro His Gly Arg Thr Gly Leu
          355          360          365
Leu Cys His Leu Asn Asp Ala Cys Ile Ser Asn Pro Cys Asn Glu Gly
          370          375          380
Ser Asn Cys Asp Thr Asn Pro Val Asn Gly Lys Ala Ile Cys Thr Cys
385          390          395          400
Pro Ser Gly Tyr Thr Gly Pro Ala Cys Ser Gln Asp Val Asp Glu Cys
          405          410          415
Ser Leu Gly Ala Asn Pro Cys Glu His Ala Gly Lys Cys Ile Asn Thr
          420          425          430
Leu Gly Ser Phe Glu Cys Gln Cys Leu Gln Gly Tyr Thr Gly Pro Arg
          435          440          445
Cys Glu Ile Asp Val Asn Glu Cys Val Ser Asn Pro Cys Gln Asn Asp
          450          455          460
Ala Thr Cys Leu Asp Gln Ile Gly Glu Phe Gln Cys Met Cys Met Pro
465          470          475          480
Gly Tyr Glu Gly Val His Cys Glu Val Asn Thr Asp Glu Cys Ala Ser
          485          490          495
Ser Pro Cys Leu His Asn Gly Arg Cys Leu Asp Lys Ile Asn Glu Phe
          500          505          510
Gln Cys Glu Cys Pro Thr Gly Phe Thr Gly His Leu Cys Gln Tyr Asp
          515          520          525
Val Asp Glu Cys Ala Ser Thr Pro Cys Lys Asn Gly Ala Lys Cys Leu
          530          535          540
Asp Gly Pro Asn Thr Tyr Thr Cys Val Cys Thr Glu Gly Tyr Thr Gly
545          550          555          560
Thr His Cys Glu Val Asp Ile Asp Glu Cys Asp Pro Asp Pro Cys His
          565          570          575
Tyr Gly Ser Cys Lys Asp Gly Val Ala Thr Phe Thr Cys Leu Cys Arg
          580          585          590
Pro Gly Tyr Thr Gly His His Cys Glu Thr Asn Ile Asn Glu Cys Ser
          595          600          605
Ser Gln Pro Cys Arg Leu Arg Gly Thr Cys Gln Asp Pro Asp Asn Ala
          610          615          620
Tyr Leu Cys Phe Cys Leu Lys Gly Thr Thr Gly Pro Asn Cys Glu Ile
625          630          635          640
Asn Leu Asp Asp Cys Ala Ser Ser Pro Cys Asp Ser Gly Thr Cys Leu
          645          650          655
Asp Lys Ile Asp Gly Tyr Glu Cys Ala Cys Glu Pro Gly Tyr Thr Gly
          660          665          670
Ser Met Cys Asn Ser Asn Ile Asp Glu Cys Ala Gly Asn Pro Cys His
          675          680          685
Asn Gly Gly Thr Cys Glu Asp Gly Ile Asn Gly Phe Thr Cys Arg Cys
          690          695          700
Pro Glu Gly Tyr His Asp Pro Thr Cys Leu Ser Glu Val Asn Glu Cys
705          710          715          720
Asn Ser Asn Pro Cys Val His Gly Ala Cys Arg Asp Ser Leu Asn Gly

```

[illegible]

- 93 -

1140	1145	1150
Ser Pro Cys Gln Asn Gly Ala Thr Cys Thr Asp Tyr Leu Gly Gly Tyr		
1155	1160	1165
Ser Cys Lys Cys Val Ala Gly Tyr His Gly Val Asn Cys Ser Glu Glu		
1170	1175	1180
Ile Asp Glu Cys Leu Ser His Pro Cys Gln Asn Gly Gly Thr Cys Leu		
1185	1190	1195
Asp Leu Pro Asn Thr Tyr Lys Cys Ser Cys Pro Arg Gly Thr Gln Gly		1200
1205	1210	1215
Val His Cys Glu Ile Asn Val Asp Asp Cys Asn Pro Pro Val Asp Pro		
1220	1225	1230
Val Ser Arg Ser Pro Lys Cys Phe Asn Asn Gly Thr Cys Val Asp Gln		
1235	1240	1245
Val Gly Gly Tyr Ser Cys Thr Cys Pro Pro Gly Phe Val Gly Glu Arg		
1250	1255	1260
Cys Glu Gly Asp Val Asn Glu Cys Leu Ser Asn Pro Cys Asp Ala Arg		
1265	1270	1275
Gly Thr Gln Asn Cys Val Gln Arg Val Asn Asp Phe His Cys Glu Cys		1280
1285	1290	1295
Arg Ala Gly His Thr Gly Arg Arg Cys Glu Ser Val Ile Asn Gly Cys		
1300	1305	1310
Lys Gly Lys Pro Cys Lys Asn Gly Gly Thr Cys Ala Val Ala Ser Asn		
1315	1320	1325
Thr Ala Arg Gly Phe Ile Cys Lys Cys Pro Ala Gly Phe Glu Gly Ala		
1330	1335	1340
Thr Cys Glu Asn Asp Ala Arg Thr Cys Gly Ser Leu Arg Cys Leu Asn		
1345	1350	1355
Gly Gly Thr Cys Ile Ser Gly Pro Arg Ser Pro Thr Cys Leu Cys Leu		1360
1365	1370	1375
Gly Pro Phe Thr Gly Pro Glu Cys Gln Phe Pro Ala Ser Ser Pro Cys		
1380	1385	1390
Leu Gly Gly Asn Pro Cys Tyr Asn Gln Gly Thr Cys Glu Pro Thr Ser		
1395	1400	1405
Glu Ser Pro Phe Tyr Arg Cys Leu Cys Pro Ala Lys Phe Asn Gly Leu		
1410	1415	1420
Leu Cys His Ile Leu Asp Tyr Ser Phe Gly Gly Gly Ala Gly Arg Asp		
1425	1430	1435
Ile Pro Pro Pro Leu Ile Glu Glu Ala Cys Glu Leu Pro Glu Cys Gln		1440
1445	1450	1455
Glu Asp Ala Gly Asn Lys Val Cys Ser Leu Gln Cys Asn Asn His Ala		
1460	1465	1470
Cys Gly Trp Asp Gly Gly Asp Cys Ser Leu Asn Phe Asn Asp Pro Trp		
1475	1480	1485
Lys Asn Cys Thr Gln Ser Leu Gln Cys Trp Lys Tyr Phe Ser Asp Gly		
1490	1495	1500
His Cys Asp Ser Gln Cys Asn Ser Ala Gly Cys Leu Phe Asp Gly Phe		
1505	1510	1515
Asp Cys Gln Arg Ala Glu Gly Gln Cys Asn Pro Leu Tyr Asp Gln Tyr		1520
1525	1530	1535
Cys Lys Asp His Phe Ser Asp Gly His Cys Asp Gln Gly Cys Asn Ser		
1540	1545	1550
Ala Glu Cys Glu Trp Asp Gly Leu Asp Cys Ala Glu His Val Pro Glu		

- 94 -

```

1555          1560          1565
Arg Leu Ala Ala Gly Thr Leu Val Val Val Val Leu Met Pro Pro Glu
1570          1575          1580
Gln Leu Arg Asn Ser Ser Phe His Phe Leu Arg Glu Leu Ser Arg Val
1585          1590          1595          1600
Leu His Thr Asn Val Val Phe Lys Arg Asp Ala His Gly Gln Gln Met
1605          1610          1615
Ile Phe Pro Tyr Tyr Gly Arg Glu Glu Glu Leu Arg Lys His Pro Ile
1620          1625          1630
Lys Arg Ala Ala Glu Gly Trp Ala Ala Pro Asp Ala Leu Leu Gly Gln
1635          1640          1645
Val Lys Ala Ser Leu Leu Pro Gly Gly Ser Glu Gly Gly Arg Arg Arg
1650          1655          1660
Arg Glu Leu Asp Pro Met Asp Val Arg Gly Ser Ile Val Tyr Leu Glu
1665          1670          1675          1680
Ile Asp Asn Arg Gln Cys Val Gln Ala Ser Ser Gln Cys Phe Gln Ser
1685          1690          1695
Ala Thr Asp Val Ala Ala Phe Leu Gly Ala Leu Ala Ser Leu Gly Ser
1700          1705          1710
Leu Asn Ile Pro Tyr Lys Ile Glu Ala Val Gln Ser Glu Thr Val Glu
1715          1720          1725
Pro Pro Pro Pro Ala Gln Leu His Phe Met Tyr Val Ala Ala Ala Ala
1730          1735          1740
Phe Val Leu Leu Phe Phe Val Gly Cys Gly Val Leu Leu Ser Arg Lys
1745          1750          1755          1760
Arg Arg Arg Gln His Gly Gln Leu Trp Phe Pro Glu Gly Phe Lys Val
1765          1770          1775
Ser Glu Ala Ser Lys Lys Lys Arg Arg Glu Pro Leu Gly Glu Asp Ser
1780          1785          1790
Val Gly Leu Lys Pro Leu Lys Asn Ala Ser Asp Gly Ala Leu Met Asp
1795          1800          1805
Asp Asn Gln Asn Glu Trp Gly Asp Glu Asp Leu Glu Thr Lys Lys Phe
1810          1815          1820
Arg Phe Glu Glu Pro Val Val Leu Pro Asp Leu Asp Asp Gln Thr Asp
1825          1830          1835          1840
His Arg Gln Trp Thr Gln Gln His Leu Asp Ala Ala Asp Leu Arg Met
1845          1850          1855
Ser Ala Met Ala Pro Thr Pro Pro Gln Gly Glu Val Asp Ala Asp Cys
1860          1865          1870
Met Asp Val Asn Val Arg Gly Pro Asp Gly Phe Thr Pro Leu Met Ile
1875          1880          1885
Ala Ser Cys Ser Gly Gly Gly Leu Glu Thr Gly Asn Ser Glu Glu Glu
1890          1895          1900
Glu Asp Ala Pro Ala Val Ile Ser Asp Phe Ile Tyr Gln Gly Ala Ser
1905          1910          1915          1920
Leu His Asn Gln Thr Asp Arg Thr Gly Glu Thr Ala Leu His Leu Ala
1925          1930          1935
Ala Arg Tyr Ser Arg Ser Asp Ala Ala Lys Arg Leu Leu Glu Ala Ser
1940          1945          1950
Ala Asp Ala Asn Ile Gln Asp Asn Met Gly Arg Thr Pro Leu His Ala
1955          1960          1965
Ala Val Ser Ala Asp Ala Gln Gly Val Phe Gln Ile Leu Ile Arg Asn

```

- 95 -

1970	1975	1980
Arg Ala Thr Asp Leu Asp Ala Arg Met His Asp Gly Thr Thr Pro Leu		
1985	1990	1995
Ile Leu Ala Ala Arg Leu Ala Val Glu Gly Met Leu Glu Asp Leu Ile		2000
	2005	2010
Asn Ser His Ala Asp Val Asn Ala Val Asp Asp Leu Gly Lys Ser Ala		2015
	2020	2025
Leu His Trp Ala Ala Ala Val Asn Asn Val Asp Ala Ala Val Val Leu		2030
	2035	2040
Leu Lys Asn Gly Ala Asn Lys Asp Met Gln Asn Asn Arg Glu Glu Thr		2045
	2050	2055
Pro Leu Phe Leu Ala Ala Arg Glu Gly Ser Tyr Glu Thr Ala Lys Val		2060
	2065	2070
Leu Leu Asp His Phe Ala Asn Arg Asp Ile Thr Asp His Met Asp Arg		2075
	2085	2090
Leu Pro Arg Asp Ile Ala Gln Glu Arg Met His His Asp Ile Val Arg		2095
	2100	2105
Leu Leu Asp Glu Tyr Asn Leu Val Arg Ser Pro Gln Leu His Gly Ala		2110
	2115	2120
Pro Leu Gly Gly Thr Pro Thr Leu Ser Pro Pro Leu Cys Ser Pro Asn		2125
	2130	2135
Gly Tyr Leu Gly Ser Leu Lys Pro Gly Val Gln Gly Lys Lys Val Arg		2140
	2145	2150
Lys Pro Ser Ser Lys Gly Leu Ala Cys Gly Ser Lys Glu Ala Lys Asp		2155
	2165	2170
Leu Lys Ala Arg Arg Lys Lys Ser Gln Asp Gly Lys Gly Cys Leu Leu		2175
	2180	2185
Asp Ser Ser Gly Met Leu Ser Pro Val Asp Ser Leu Glu Ser Pro His		2190
	2195	2200
Gly Tyr Leu Ser Asp Val Ala Ser Pro Pro Leu Leu Pro Ser Pro Phe		2205
	2210	2215
Gln Gln Ser Pro Ser Val Pro Leu Asn His Leu Pro Gly Met Pro Asp		2220
	2225	2230
Thr His Leu Gly Ile Gly His Leu Asn Val Ala Ala Lys Pro Glu Met		2235
	2245	2250
Ala Ala Leu Gly Gly Gly Arg Leu Ala Phe Glu Thr Gly Pro Pro		2255
	2260	2265
Arg Leu Ser His Leu Pro Val Ala Ser Gly Thr Ser Thr Val Leu Gly		2270
	2275	2280
Ser Ser Ser Gly Gly Ala Leu Asn Phe Thr Val Gly Gly Ser Thr Ser		2285
	2290	2295
Leu Asn Gly Gln Cys Glu Trp Leu Ser Arg Leu Gln Ser Gly Met Val		2300
	2305	2310
Pro Asn Gln Tyr Asn Pro Leu Arg Gly Ser Val Ala Pro Gly Pro Leu		2315
	2325	2330
Ser Thr Gln Ala Pro Ser Leu Gln His Gly Met Val Gly Pro Leu His		2335
	2340	2345
Ser Ser Leu Ala Ala Ser Ala Leu Ser Gln Met Met Ser Tyr Gln Gly		2350
	2355	2360
Leu Pro Ser Thr Arg Leu Ala Thr Gln Pro His Leu Val Gln Thr Gln		2365
	2370	2375
Gln Val Gln Pro Gln Asn Leu Gln Met Gln Gln Gln Asn Leu Gln Pro		2380

- 96 -

```

2385          2390          2395          2400
Ala Asn Ile Gln Gln Gln Gln Ser Leu Gln Pro Pro Pro Pro Pro Pro
          2405          2410          2415
Gln Pro His Leu Gly Val Ser Ser Ala Ala Ser Gly His Leu Gly Arg
          2420          2425          2430
Ser Phe Leu Ser Gly Glu Pro Ser Gln Ala Asp Val Gln Pro Leu Gly
          2435          2440          2445
Pro Ser Ser Leu Ala Val His Thr Ile Leu Pro Gln Glu Ser Pro Ala
          2450          2455          2460
Leu Pro Thr Ser Leu Pro Ser Ser Leu Val Pro Pro Val Thr Ala Ala
2465          2470          2475          2480
Gln Phe Leu Thr Pro Pro Ser Gln His Ser Tyr Ser Ser Pro Val Asp
          2485          2490          2495
Asn Thr Pro Ser His Gln Leu Gln Val Pro Glu His Pro Phe Leu Thr
          2500          2505          2510
Pro Ser Pro Glu Ser Pro Asp Gln Trp Ser Ser Ser Ser Pro His Ser
          2515          2520          2525
Asn Val Ser Asp Trp Ser Glu Gly Val Ser Ser Pro Pro Thr Ser Met
          2530          2535          2540
Gln Ser Gln Ile Ala Arg Ile Pro Glu Ala Phe Lys
2545          2550          2555

```

```

<210> 33
<211> 2649
<212> DNA
<213> Homo Sapiens

```

```

<220>
<221> CDS
<222> (1)...(2649)
<223> E-Cadherin

```

```

<300>
<308> Genbank NM_004360
<309> 2001-07-03

```

```

<400> 33
atg ggc cct tgg agc cgc agc ctc tgc gcg ctg ctg ctg ctg ctg cag   48
Met Gly Pro Trp Ser Arg Ser Leu Ser Ala Leu Leu Leu Leu Leu Gln
  1              5              10              15

gtc tcc tct tgg ctc tgc cag gag ccg gag ccc tgc cac cct ggc ttt   96
Val Ser Ser Trp Leu Cys Gln Glu Pro Glu Pro Cys His Pro Gly Phe
      20              25              30

gac gcc gag agc tac acg ttc acg gtg ccc cgg cgc cac ctg gag aga   144
Asp Ala Glu Ser Tyr Thr Phe Thr Val Pro Arg Arg His Leu Glu Arg
      35              40              45

ggc cgc gtc ctg ggc aga gtg aat ttt gaa gat tgc acc ggt cga caa   192
Gly Arg Val Leu Gly Arg Val Asn Phe Glu Asp Cys Thr Gly Arg Gln

```

- 97 -

50	55	60	
agg aca gcc tat ttt tcc ctc gac acc cga ttc aaa gtg ggc aca gat			240
Arg Thr Ala Tyr Phe Ser Leu Asp Thr Arg Phe Lys Val Gly Thr Asp			
65	70	75	80
ggt gtg att aca gtc aaa agg cct cta cgg ttt cat aac cca cag atc			288
Gly Val Ile Thr Val Lys Arg Pro Leu Arg Phe His Asn Pro Gln Ile			
	85	90	95
cat ttc ttg gtc tac gcc tgg gac tcc acc tac aga aag ttt tcc acc			336
His Phe Leu Val Tyr Ala Trp Asp Ser Thr Tyr Arg Lys Phe Ser Thr			
	100	105	110
aaa gtc acg ctg aat aca gtg ggg cac cac cac cgc ccc ccg ccc cat			384
Lys Val Thr Leu Asn Thr Val Gly His His His Arg Pro Pro Pro His			
	115	120	125
cag gcc tcc gtt tct gga atc caa gca gaa ttg ctc aca ttt ccc aac			432
Gln Ala Ser Val Ser Gly Ile Gln Ala Glu Leu Leu Thr Phe Pro Asn			
	130	135	140
tcc tct cct ggc ctc aga aga cag aag aga gac tgg gtt att cct ccc			480
Ser Ser Pro Gly Leu Arg Arg Gln Lys Arg Asp Trp Val Ile Pro Pro			
145	150	155	160
atc agc tgc cca gaa aat gaa aaa ggc cca ttt cct aaa aac ctg gtt			528
Ile Ser Cys Pro Glu Asn Glu Lys Gly Pro Phe Pro Lys Asn Leu Val			
	165	170	175
cag atc aaa tcc aac aaa gac aaa gaa ggc aag gtt ttc tac agc atc			576
Gln Ile Lys Ser Asn Lys Asp Lys Glu Gly Lys Val Phe Tyr Ser Ile			
	180	185	190
act ggc caa gga gct gac aca ccc cct gtt ggt gtc ttt att att gaa			624
Thr Gly Gln Gly Ala Asp Thr Pro Pro Val Gly Val Phe Ile Ile Glu			
	195	200	205
aga gaa aca gga tgg ctg aag gtg aca gag cct ctg gat aga gaa cgc			672
Arg Glu Thr Gly Trp Leu Lys Val Thr Glu Pro Leu Asp Arg Glu Arg			
	210	215	220
att gcc aca tac act ctc ttc tct cac gct gtg tca tcc aac ggg aat			720
Ile Ala Thr Tyr Thr Leu Phe Ser His Ala Val Ser Ser Asn Gly Asn			
225	230	235	240
gca gtt gag gat cca atg gag att ttg atc acg gta acc gat cag aat			768
Ala Val Glu Asp Pro Met Glu Ile Leu Ile Thr Val Thr Asp Gln Asn			
	245	250	255
gac aac aag ccc gaa ttc acc cag gag gtc ttt aag ggg tct gtc atg			816
Asp Asn Lys Pro Glu Phe Thr Gln Glu Val Phe Lys Gly Ser Val Met			

- 98 -

260	265	270	
gaa ggt gct ctt cca gga acc tct gtg atg gag gtc aca gcc aca gac Glu Gly Ala Leu Pro Gly Thr Ser Val Met Glu Val Thr Ala Thr Asp 275 280 285			864
gcg gac gat gat gtg aac acc tac aat gcc gcc atc gct tac acc atc Ala Asp Asp Asp Val Asn Thr Tyr Asn Ala Ala Ile Ala Tyr Thr Ile 290 295 300			912
ctc agc caa gat cct gag ctc cct gac aaa aat atg ttc acc att aac Leu Ser Gln Asp Pro Glu Leu Pro Asp Lys Asn Met Phe Thr Ile Asn 305 310 315 320			960
agg aac aca gga gtc atc agt gtg gtc acc act ggg ctg gac cga gag Arg Asn Thr Gly Val Ile Ser Val Val Thr Thr Gly Leu Asp Arg Glu 325 330 335			1008
agt ttc cct acg tat acc ctg gtg gtt caa gct gct gac ctt caa ggt Ser Phe Pro Thr Tyr Thr Leu Val Val Gln Ala Ala Asp Leu Gln Gly 340 345 350			1056
gag ggg tta agc aca aca gca aca gct gtg atc aca gtc act gac acc Glu Gly Leu Ser Thr Thr Ala Thr Ala Val Ile Thr Val Thr Asp Thr 355 360 365			1104
aac gat aat cct ccg atc ttc aat ccc acc acg tac aag ggt cag gtg Asn Asp Asn Pro Pro Ile Phe Asn Pro Thr Thr Tyr Lys Gly Gln Val 370 375 380			1152
cct gag aac gag gct aac gtc gta atc acc aca ctg aaa gtg act gat Pro Glu Asn Glu Ala Asn Val Val Ile Thr Thr Leu Lys Val Thr Asp 385 390 395 400			1200
gct gat gcc ccc aat acc cca gcg tgg gag gct gta tac acc ata ttg Ala Asp Ala Pro Asn Thr Pro Ala Trp Glu Ala Val Tyr Thr Ile Leu 405 410 415			1248
aat gat gat ggt gga caa ttt gtc gtc acc aca aat cca gtg aac aac Asn Asp Asp Gly Gly Gln Phe Val Val Thr Thr Asn Pro Val Asn Asn 420 425 430			1296
gat ggc att ttg aaa aca gca aag ggc ttg gat ttt gag gcc aag cag Asp Gly Ile Leu Lys Thr Ala Lys Gly Leu Asp Phe Glu Ala Lys Gln 435 440 445			1344
cag tac att cta cac gta gca gtg acg aat gtg gta cct ttt gag gtc Gln Tyr Ile Leu His Val Ala Val Thr Asn Val Val Pro Phe Glu Val 450 455 460			1392
tct ctc acc acc tcc aca gcc acc gtc acc gtg gat gtg ctg gat gtg Ser Leu Thr Thr Ser Thr Ala Thr Val Thr Val Asp Val Leu Asp Val 465 470 475 480			1440

- 99 -

465	470	475	480	
aat gaa gcc ccc atc ttt gtg cct cct gaa aag aga gtg gaa gtg tcc				1488
Asn Glu Ala Pro Ile Phe Val Pro Pro Glu Lys Arg Val Glu Val Ser				
	485	490	495	
gag gac ttt ggc gtg ggc cag gaa atc aca tcc tac act gcc cag gag				1536
Glu Asp Phe Gly Val Gly Gln Glu Ile Thr Ser Tyr Thr Ala Gln Glu				
	500	505	510	
cca gac aca ttt atg gaa cag aaa ata aca tat cgg att tgg aga gac				1584
Pro Asp Thr Phe Met Glu Gln Lys Ile Thr Tyr Arg Ile Trp Arg Asp				
	515	520	525	
act gcc aac tgg ctg gag att aat ccg gac act ggt gcc att tcc act				1632
Thr Ala Asn Trp Leu Glu Ile Asn Pro Asp Thr Gly Ala Ile Ser Thr				
	530	535	540	
cgg gct gag ctg gac agg gag gat ttt gag cac gtg aag aac agc acg				1680
Arg Ala Glu Leu Asp Arg Glu Asp Phe Glu His Val Lys Asn Ser Thr				
	545	550	555	560
tac aca gcc cta atc ata gct aca gac aat ggt tct cca gtt gct act				1728
Tyr Thr Ala Leu Ile Ile Ala Thr Asp Asn Gly Ser Pro Val Ala Thr				
	565	570	575	
gga aca ggg aca ctt ctg ctg atc ctg tct gat gtg aat gac aac gcc				1776
Gly Thr Gly Thr Leu Leu Leu Ile Leu Ser Asp Val Asn Asp Asn Ala				
	580	585	590	
ccc ata cca gaa cct cga act ata ttc ttc tgt gag agg aat cca aag				1824
Pro Ile Pro Glu Pro Arg Thr Ile Phe Phe Cys Glu Arg Asn Pro Lys				
	595	600	605	
cct cag gtc ata aac atc att gat gca gac ctt cct ccc aat aca tct				1872
Pro Gln Val Ile Asn Ile Ile Asp Ala Asp Leu Pro Pro Asn Thr Ser				
	610	615	620	
ccc ttc aca gca gaa cta aca cac ggg gcg agt gcc aac tgg acc att				1920
Pro Phe Thr Ala Glu Leu Thr His Gly Ala Ser Ala Asn Trp Thr Ile				
	625	630	635	640
cag tac aac gac cca acc caa gaa tct atc att ttg aag cca aag atg				1968
Gln Tyr Asn Asp Pro Thr Gln Glu Ser Ile Ile Leu Lys Pro Lys Met				
	645	650	655	
gcc tta gag gtg ggt gac tac aaa atc aat ctc aag ctc atg gat aac				2016
Ala Leu Glu Val Gly Asp Tyr Lys Ile Asn Leu Lys Leu Met Asp Asn				
	660	665	670	
cag aat aaa gac caa gtg acc acc tta gag gtc agc gtg tgt gac tgt				2064
Gln Asn Lys Asp Gln Val Thr Thr Leu Glu Val Ser Val Cys Asp Cys				

- 100 -

675	680	685	
gaa ggg gcc gcc ggc gtc	tgt agg aag gca cag cct gtc	gaa gca gga	2112
Glu Gly Ala Ala Gly Val	Cys Arg Lys Ala Gln Pro Val	Glu Ala Gly	
690	695	700	
ttg caa att cct gcc att	ctg ggg att ctt gga gga att	ctt gct ttg	2160
Leu Gln Ile Pro Ala Ile	Leu Gly Ile Leu Gly Ile	Leu Ala Leu	
705	710	715 720	
cta att ctg att ctg ctg	ctc ttg ctg ttt ctt cgg agg	aga gcg gtg	2208
Leu Ile Leu Ile Leu Leu	Leu Leu Leu Phe Leu Arg	Arg Arg Ala Val	
725	730	735	
gtc aaa gag ccc tta ctg	ccc cca gag gat gac acc	cgg gac aac gtt	2256
Val Lys Glu Pro Leu Leu	Pro Pro Glu Asp Asp Thr	Arg Asp Asn Val	
740	745	750	
tat tac tat gat gaa gaa	gga ggc gga gaa gag gac	cag gac ttt gac	2304
Tyr Tyr Tyr Asp Glu Glu	Gly Gly Gly Glu Glu Asp	Gln Asp Phe Asp	
755	760	765	
ttg agc cag ctg cac agg	ggc ctg gac gct cgg cct	gaa gtg act cgt	2352
Leu Ser Gln Leu His Arg	Gly Leu Asp Ala Arg Pro	Glu Val Thr Arg	
770	775	780	
aac gac gtt gca cca acc	ctc atg agt gtc ccc cgg	tat ctt ccc cgc	2400
Asn Asp Val Ala Pro Thr	Leu Met Ser Val Pro Arg	Tyr Leu Pro Arg	
785	790	795 800	
cct gcc aat ccc gat gaa	att gga aat ttt att gat	gaa aat ctg aaa	2448
Pro Ala Asn Pro Asp Glu	Ile Gly Asn Phe Ile Asp	Glu Asn Leu Lys	
805	810	815	
gcg gct gat act gac ccc	aca gcc ccg cct tat gat	tct ctg ctc gtg	2496
Ala Ala Asp Thr Asp Pro	Thr Ala Pro Pro Tyr Asp	Ser Leu Leu Val	
820	825	830	
ttt gac tat gaa gga agc	ggt tcc gaa gct gct agt	ctg agc tcc ctg	2544
Phe Asp Tyr Glu Gly Ser	Gly Ser Glu Ala Ala Ser	Leu Ser Ser Leu	
835	840	845	
aac tcc tca gag tca gac	aaa gac cag gac tat gac	tac ttg aac gaa	2592
Asn Ser Ser Glu Ser Asp	Lys Asp Gln Asp Tyr Asp	Tyr Leu Asn Glu	
850	855	860	
tgg ggc aat cgc ttc aag	aag ctg gct gac atg tac	gga ggc ggc gag	2640
Trp Gly Asn Arg Phe Lys	Lys Leu Ala Asp Met Tyr	Gly Gly Gly Glu	
865	870	875 880	
gac gac tag			2649
Asp Asp			

- 101 -

<210> 34
 <211> 882
 <212> PRT
 <213> Homo Sapiens

<300>
 <308> Genbank NP_004351
 <309> 2001-07-03

<400> 34
 Met Gly Pro Trp Ser Arg Ser Leu Ser Ala Leu Leu Leu Leu Leu Gln
 1 5 10 15
 Val Ser Ser Trp Leu Cys Gln Glu Pro Glu Pro Cys His Pro Gly Phe
 20 25 30
 Asp Ala Glu Ser Tyr Thr Phe Thr Val Pro Arg Arg His Leu Glu Arg
 35 40 45
 Gly Arg Val Leu Gly Arg Val Asn Phe Glu Asp Cys Thr Gly Arg Gln
 50 55 60
 Arg Thr Ala Tyr Phe Ser Leu Asp Thr Arg Phe Lys Val Gly Thr Asp
 65 70 75 80
 Gly Val Ile Thr Val Lys Arg Pro Leu Arg Phe His Asn Pro Gln Ile
 85 90 95
 His Phe Leu Val Tyr Ala Trp Asp Ser Thr Tyr Arg Lys Phe Ser Thr
 100 105 110
 Lys Val Thr Leu Asn Thr Val Gly His His His Arg Pro Pro Pro His
 115 120 125
 Gln Ala Ser Val Ser Gly Ile Gln Ala Glu Leu Leu Thr Phe Pro Asn
 130 135 140
 Ser Ser Pro Gly Leu Arg Arg Gln Lys Arg Asp Trp Val Ile Pro Pro
 145 150 155 160
 Ile Ser Cys Pro Glu Asn Glu Lys Gly Pro Phe Pro Lys Asn Leu Val
 165 170 175
 Gln Ile Lys Ser Asn Lys Asp Lys Glu Gly Lys Val Phe Tyr Ser Ile
 180 185 190
 Thr Gly Gln Gly Ala Asp Thr Pro Pro Val Gly Val Phe Ile Ile Glu
 195 200 205
 Arg Glu Thr Gly Trp Leu Lys Val Thr Glu Pro Leu Asp Arg Glu Arg
 210 215 220
 Ile Ala Thr Tyr Thr Leu Phe Ser His Ala Val Ser Ser Asn Gly Asn
 225 230 235 240
 Ala Val Glu Asp Pro Met Glu Ile Leu Ile Thr Val Thr Asp Gln Asn
 245 250 255
 Asp Asn Lys Pro Glu Phe Thr Gln Glu Val Phe Lys Gly Ser Val Met
 260 265 270
 Glu Gly Ala Leu Pro Gly Thr Ser Val Met Glu Val Thr Ala Thr Asp
 275 280 285
 Ala Asp Asp Asp Val Asn Thr Tyr Asn Ala Ala Ile Ala Tyr Thr Ile
 290 295 300
 Leu Ser Gln Asp Pro Glu Leu Pro Asp Lys Asn Met Phe Thr Ile Asn

- 102 -

```

305          310          315          320
Arg Asn Thr Gly Val Ile Ser Val Val Thr Thr Gly Leu Asp Arg Glu
          325          330          335
Ser Phe Pro Thr Tyr Thr Leu Val Val Gln Ala Ala Asp Leu Gln Gly
          340          345          350
Glu Gly Leu Ser Thr Thr Ala Thr Ala Val Ile Thr Val Thr Asp Thr
          355          360          365
Asn Asp Asn Pro Pro Ile Phe Asn Pro Thr Thr Tyr Lys Gly Gln Val
          370          375          380
Pro Glu Asn Glu Ala Asn Val Val Ile Thr Thr Leu Lys Val Thr Asp
385          390          395          400
Ala Asp Ala Pro Asn Thr Pro Ala Trp Glu Ala Val Tyr Thr Ile Leu
          405          410          415
Asn Asp Asp Gly Gly Gln Phe Val Val Thr Thr Asn Pro Val Asn Asn
          420          425          430
Asp Gly Ile Leu Lys Thr Ala Lys Gly Leu Asp Phe Glu Ala Lys Gln
          435          440          445
Gln Tyr Ile Leu His Val Ala Val Thr Asn Val Val Pro Phe Glu Val
          450          455          460
Ser Leu Thr Thr Ser Thr Ala Thr Val Thr Val Asp Val Leu Asp Val
465          470          475          480
Asn Glu Ala Pro Ile Phe Val Pro Pro Glu Lys Arg Val Glu Val Ser
          485          490          495
Glu Asp Phe Gly Val Gly Gln Glu Ile Thr Ser Tyr Thr Ala Gln Glu
          500          505          510
Pro Asp Thr Phe Met Glu Gln Lys Ile Thr Tyr Arg Ile Trp Arg Asp
          515          520          525
Thr Ala Asn Trp Leu Glu Ile Asn Pro Asp Thr Gly Ala Ile Ser Thr
          530          535          540
Arg Ala Glu Leu Asp Arg Glu Asp Phe Glu His Val Lys Asn Ser Thr
545          550          555          560
Tyr Thr Ala Leu Ile Ile Ala Thr Asp Asn Gly Ser Pro Val Ala Thr
          565          570          575
Gly Thr Gly Thr Leu Leu Leu Ile Leu Ser Asp Val Asn Asp Asn Ala
          580          585          590
Pro Ile Pro Glu Pro Arg Thr Ile Phe Phe Cys Glu Arg Asn Pro Lys
          595          600          605
Pro Gln Val Ile Asn Ile Ile Asp Ala Asp Leu Pro Pro Asn Thr Ser
          610          615          620
Pro Phe Thr Ala Glu Leu Thr His Gly Ala Ser Ala Asn Trp Thr Ile
625          630          635          640
Gln Tyr Asn Asp Pro Thr Gln Glu Ser Ile Ile Leu Lys Pro Lys Met
          645          650          655
Ala Leu Glu Val Gly Asp Tyr Lys Ile Asn Leu Lys Leu Met Asp Asn
          660          665          670
Gln Asn Lys Asp Gln Val Thr Thr Leu Glu Val Ser Val Cys Asp Cys
          675          680          685
Glu Gly Ala Ala Gly Val Cys Arg Lys Ala Gln Pro Val Glu Ala Gly
          690          695          700
Leu Gln Ile Pro Ala Ile Leu Gly Ile Leu Gly Gly Ile Leu Ala Leu
705          710          715          720
Leu Ile Leu Ile Leu Leu Leu Leu Phe Leu Arg Arg Arg Ala Val

```

				725				730				735			
Val	Lys	Glu	Pro	Leu	Leu	Pro	Pro	Glu	Asp	Asp	Thr	Arg	Asp	Asn	Val
				740				745				750			
Tyr	Tyr	Tyr	Asp	Glu	Glu	Gly	Gly	Gly	Glu	Glu	Asp	Gln	Asp	Phe	Asp
				755				760				765			
Leu	Ser	Gln	Leu	His	Arg	Gly	Leu	Asp	Ala	Arg	Pro	Glu	Val	Thr	Arg
				770				775				780			
Asn	Asp	Val	Ala	Pro	Thr	Leu	Met	Ser	Val	Pro	Arg	Tyr	Leu	Pro	Arg
				785				790				795			
Pro	Ala	Asn	Pro	Asp	Glu	Ile	Gly	Asn	Phe	Ile	Asp	Glu	Asn	Leu	Lys
				805				810				815			
Ala	Ala	Asp	Thr	Asp	Pro	Thr	Ala	Pro	Pro	Tyr	Asp	Ser	Leu	Leu	Val
				820				825				830			
Phe	Asp	Tyr	Glu	Gly	Ser	Gly	Ser	Glu	Ala	Ala	Ser	Leu	Ser	Ser	Leu
				835				840				845			
Asn	Ser	Ser	Glu	Ser	Asp	Lys	Asp	Gln	Asp	Tyr	Asp	Tyr	Leu	Asn	Glu
				850				855				860			
Trp	Gly	Asn	Arg	Phe	Lys	Lys	Leu	Ala	Asp	Met	Tyr	Gly	Gly	Gly	Glu
				865				870				875			
Asp Asp												880			

```
<220>
<221> CDS
<222> (34)...(3960)
<223> Receptor Tyrosine Kinase (ERBB4)
```

```

<400> 35
aattgtcagc acgggatctg agacttccaa aaa atg aag ccg gcg aca gga ctt 54
                               Met Lys Pro Ala Thr Gly Leu
                               1                               5

tgg gtc tgg gtg agc ctt ctc gtg gcg gcg ggg acc gtc cag ccc agc 102
Trp Val Trp Val Ser Leu Leu Val Ala Ala Gly Thr Val Gln Pro Ser
      10                               15                               20

gat tct cag tca gtg tgt gca gga acg gag aat aaa ctg agc tct ctc 150
Asp Ser Gln Ser Val Cys Ala Gly Thr Glu Asn Lys Leu Ser Ser Leu
      25                               30                               35

tct gac ctg gaa cag cag tac cga gcc ttg cgc aag tac tat gaa aac 198
Ser Asp Leu Glu Gln Gln Tyr Arg Ala Leu Arg Lys Tyr Tyr Glu Asn

```

- 104 -

40	45	50	55	
tgt gag gtt gtc atg ggc aac ctg gag ata acc agc att gag cac aac				246
Cys Glu Val Val Met Gly Asn Leu Glu Ile Thr Ser Ile Glu His Asn				
	60	65	70	
cgg gac ctc tcc ttc ctg cgg tct gtt cga gaa gtc aca ggc tac gtg				294
Arg Asp Leu Ser Phe Leu Arg Ser Val Arg Glu Val Thr Gly Tyr Val				
	75	80	85	
tta gtg gct ctt aat cag ttt cgt tac ctg cct ctg gag aat tta cgc				342
Leu Val Ala Leu Asn Gln Phe Arg Tyr Leu Pro Leu Glu Asn Leu Arg				
	90	95	100	
att att cgt ggg aca aaa ctt tat gag gat cga tat gcc ttg gca ata				390
Ile Ile Arg Gly Thr Lys Leu Tyr Glu Asp Arg Tyr Ala Leu Ala Ile				
	105	110	115	
ttt tta aac tac aga aaa gat gga aac ttt gga ctt caa gaa ctt gga				438
Phe Leu Asn Tyr Arg Lys Asp Gly Asn Phe Gly Leu Gln Glu Leu Gly				
	120	125	130	135
tta aag aac ttg aca gaa atc cta aat ggt gga gtc tat gta gac cag				486
Leu Lys Asn Leu Thr Glu Ile Leu Asn Gly Gly Val Tyr Val Asp Gln				
	140	145	150	
aac aaa ttc ctt tgt tat gca gac acc att cat tgg caa gat att gtt				534
Asn Lys Phe Leu Cys Tyr Ala Asp Thr Ile His Trp Gln Asp Ile Val				
	155	160	165	
cgg aac cca tgg cct tcc aac ttg act ctt gtg tca aca aat ggt agt				582
Arg Asn Pro Trp Pro Ser Asn Leu Thr Leu Val Ser Thr Asn Gly Ser				
	170	175	180	
tca gga tgt gga cgt tgc cat aag tcc tgt act ggc cgt tgc tgg gga				630
Ser Gly Cys Gly Arg Cys His Lys Ser Cys Thr Gly Arg Cys Trp Gly				
	185	190	195	
ccc aca gaa aat cat tgc cag act ttg aca agg acg gtg tgt gca gaa				678
Pro Thr Glu Asn His Cys Gln Thr Leu Thr Arg Thr Val Cys Ala Glu				
	200	205	210	215
caa tgt gac ggc aga tgc tac gga cct tac gtc agt gac tgc tgc cat				726
Gln Cys Asp Gly Arg Cys Tyr Gly Pro Tyr Val Ser Asp Cys Cys His				
	220	225	230	
cga gaa tgt gct gga ggc tgc tca gga cct aag gac aca gac tgc ttt				774
Arg Glu Cys Ala Gly Gly Cys Ser Gly Pro Lys Asp Thr Asp Cys Phe				
	235	240	245	
gcc tgc atg aat ttc aat gac agt gga gca tgt gtt act cag tgt ccc				822
Ala Cys Met Asn Phe Asn Asp Ser Gly Ala Cys Val Thr Gln Cys Pro				

- 105 -

250	255	260	
caa acc ttt gtc tac aat cca acc acc ttt	caa ctg gag cac aat ttc	870	
Gln Thr Phe Val Tyr Asn Pro Thr Thr Phe	Gln Leu Glu His Asn Phe		
265	270 275		
aat gca aag tac aca tat gga gca ttc tgt gtc aag aaa tgt cca cat	918		
Asn Ala Lys Tyr Thr Tyr Gly Ala Phe Cys Val Lys Lys Cys Pro His			
280	285 290 295		
aac ttt gtg gta gat tcc agt tct tgt gtg cgt gcc tgc cct agt tcc	966		
Asn Phe Val Val Asp Ser Ser Ser Cys Val Arg Ala Cys Pro Ser Ser			
	300 305 310		
aag atg gaa gta gaa gaa aat ggg att aaa atg tgt aaa cct tgc act	1014		
Lys Met Glu Val Glu Glu Asn Gly Ile Lys Met Cys Lys Pro Cys Thr			
	315 320 325		
gac att tgc cca aaa gct tgt gat ggc att ggc aca gga tca ttg atg	1062		
Asp Ile Cys Pro Lys Ala Cys Asp Gly Ile Gly Thr Gly Ser Leu Met			
	330 335 340		
tca gct cag act gtg gat tcc agt aac att gac aaa ttc ata aac tgt	1110		
Ser Ala Gln Thr Val Asp Ser Ser Asn Ile Asp Lys Phe Ile Asn Cys			
	345 350 355		
acc aag atc aat ggg aat ttg atc ttt cta gtc act ggt att cat ggg	1158		
Thr Lys Ile Asn Gly Asn Leu Ile Phe Leu Val Thr Gly Ile His Gly			
	360 365 370 375		
gac cct tac aat gca att gaa gcc ata gac cca gag aaa ctg aac gtc	1206		
Asp Pro Tyr Asn Ala Ile Glu Ala Ile Asp Pro Glu Lys Leu Asn Val			
	380 385 390		
ttt cgg aca gtc aga gag ata aca ggt ttc ctg aac ata cag tca tgg	1254		
Phe Arg Thr Val Arg Glu Ile Thr Gly Phe Leu Asn Ile Gln Ser Trp			
	395 400 405		
cca cca aac atg act gac ttc agt gtt ttt tct aac ctg gtg acc att	1302		
Pro Pro Asn Met Thr Asp Phe Ser Val Phe Ser Asn Leu Val Thr Ile			
	410 415 420		
ggt gga aga gta ctc tat agt ggc ctg tcc ttg ctt atc ctc aag caa	1350		
Gly Gly Arg Val Leu Tyr Ser Gly Leu Ser Leu Leu Ile Leu Lys Gln			
	425 430 435		
cag ggc atc acc tct cta cag ttc cag tcc ctg aag gaa atc agc gca	1398		
Gln Gly Ile Thr Ser Leu Gln Phe Gln Ser Leu Lys Glu Ile Ser Ala			
	440 445 450 455		
gga aac atc tat att act gac aac agc aac ctg tgt tat tat cat acc	1446		
Gly Asn Ile Tyr Ile Thr Asp Asn Ser Asn Leu Cys Tyr Tyr His Thr			

- 106 -

460	465	470	
att aac tgg aca aca ctc ttc agc	aca atc aac cag aga ata gta atc		1494
Ile Asn Trp Thr Thr Leu Phe Ser Thr	Ile Asn Gln Arg Ile Val Ile		
475	480	485	
cgg gac aac aga aaa gct gaa aat	tgt act gct gaa gga atg gtg tgc		1542
Arg Asp Asn Arg Lys Ala Glu Asn Cys	Thr Ala Glu Gly Met Val Cys		
490	495	500	
aac cat ctg tgt tcc agt gat ggc	tgt tgg gga cct ggg cca gac caa		1590
Asn His Leu Cys Ser Ser Asp Gly Cys	Trp Gly Pro Gly Pro Asp Gln		
505	510	515	
tgt ctg tgg tgt cgc cgc ttc agt	aga gga agg atc tgc ata gag tct		1638
Cys Leu Ser Cys Arg Arg Phe Ser Arg	Gly Arg Ile Cys Ile Glu Ser		
520	525	530	535
tgt aac ctc tat gat ggt gaa ttt	cgg gag ttt gag aat ggc tcc atc		1686
Cys Asn Leu Tyr Asp Gly Glu Phe Arg	Glu Phe Glu Asn Gly Ser Ile		
540	545	550	
tgt gtg gag tgt gac ccc cag tgt	gag aag atg gaa gat ggc ctc ctc		1734
Cys Val Glu Cys Asp Pro Gln Cys Glu	Lys Met Glu Asp Gly Leu Leu		
555	560	565	
aca tgc cat gga ccg ggt cct gac	aac tgt aca aag tgc tct cat ttt		1782
Thr Cys His Gly Pro Gly Pro Asp Asn	Cys Thr Lys Cys Ser His Phe		
570	575	580	
aaa gat ggc cca aac tgt gtg gaa	aaa tgt cca gat ggc tta cag ggg		1830
Lys Asp Gly Pro Asn Cys Val Glu Lys	Cys Pro Asp Gly Leu Gln Gly		
585	590	595	
gca aac agt ttc att ttc aag tat	gct gat cca gat cgg gag tgc cac		1878
Ala Asn Ser Phe Ile Phe Lys Tyr Ala	Asp Pro Asp Arg Glu Cys His		
600	605	610	615
cca tgc cat cca aac tgc acc caa	ggg tgt aac ggt ccc act agt cat		1926
Pro Cys His Pro Asn Cys Thr Gln Gly	Cys Asn Gly Pro Thr Ser His		
620	625	630	
gac tgc att tac tac cca tgg acg	ggc cat tcc act tta cca caa cat		1974
Asp Cys Ile Tyr Tyr Pro Trp Thr Gly	His Ser Thr Leu Pro Gln His		
635	640	645	
gct aga act ccc ctg att gca gct	gga gta att ggt ggg ctc ttc att		2022
Ala Arg Thr Pro Leu Ile Ala Ala Gly	Val Ile Gly Gly Leu Phe Ile		
650	655	660	
ctg gtc att gtg ggt ctg aca ttt	gct gtt tat gtt aga agg aag agc		2070
Leu Val Ile Val Gly Leu Thr Phe Ala	Val Tyr Val Arg Arg Lys Ser		

- 107 -

665	670	675	
atc aaa aag aaa aga gcc ttg aga aga ttc ttg gaa aca gag ttg gtg			2118
Ile Lys Lys Lys Arg Ala Leu Arg Arg Phe Leu Glu Thr Glu Leu Val			
680	685	690	695
gaa cca tta act ccc agt ggc aca gca ccc aat caa gct caa ctt cgt			2166
Glu Pro Leu Thr Pro Ser Gly Thr Ala Pro Asn Gln Ala Gln Leu Arg			
	700	705	710
att ttg aaa gaa act gag ctg aag agg gta aaa gtc ctt ggc tca ggt			2214
Ile Leu Lys Glu Thr Glu Leu Lys Arg Val Lys Val Leu Gly Ser Gly			
	715	720	725
gct ttt gga acg gtt tat aaa ggt att tgg gta cct gaa gga gaa act			2262
Ala Phe Gly Thr Val Tyr Lys Gly Ile Trp Val Pro Glu Gly Glu Thr			
	730	735	740
gtg aag att cct gtg gct att aag att ctt aat gag aca act ggt ccc			2310
Val Lys Ile Pro Val Ala Ile Lys Ile Leu Asn Glu Thr Thr Gly Pro			
	745	750	755
aag gca aat gtg gag ttc atg gat gaa gct ctg atc atg gca agt atg			2358
Lys Ala Asn Val Glu Phe Met Asp Glu Ala Leu Ile Met Ala Ser Met			
	760	765	770
gat cat cca cac cta gtc cgg ttg ctg ggt gtg tgt ctg agc cca acc			2406
Asp His Pro His Leu Val Arg Leu Leu Gly Val Cys Leu Ser Pro Thr			
	780	785	790
atc cag ctg gtt act caa ctt atg ccc cat ggc tgc ctg ttg gag tat			2454
Ile Gln Leu Val Thr Gln Leu Met Pro His Gly Cys Leu Leu Glu Tyr			
	795	800	805
gtc cac gag cac aag gat aac att gga tca caa ctg ctg ctt aac tgg			2502
Val His Glu His Lys Asp Asn Ile Gly Ser Gln Leu Leu Leu Asn Trp			
	810	815	820
tgt gtc cag ata gct aag gga atg atg tac ctg gaa gaa aga cga ctc			2550
Cys Val Gln Ile Ala Lys Gly Met Met Tyr Leu Glu Glu Arg Arg Leu			
	825	830	835
gtt cat cgg gat ttg gca gcc cgt aat gtc tta gtg aaa tct cca aac			2598
Val His Arg Asp Leu Ala Ala Arg Asn Val Leu Val Lys Ser Pro Asn			
	840	845	850
cat gtg aaa atc aca gat ttt ggg cta gcc aga ctc ttg gaa gga gat			2646
His Val Lys Ile Thr Asp Phe Gly Leu Ala Arg Leu Leu Glu Gly Asp			
	860	865	870
gaa aaa gag tac aat gct gat gga gga aag atg cca att aaa tgg atg			2694
Glu Lys Glu Tyr Asn Ala Asp Gly Gly Lys Met Pro Ile Lys Trp Met			

- 108 -

875	880	885	
gct ctg gag tgt ata cat tac agg	aaa ttc acc cat	cag agt gac gtt	2742
Ala Leu Glu Cys Ile His Tyr Arg	Lys Phe Thr His	Gln Ser Asp Val	
890	895	900	
tgg agc tat gga gtt act ata tgg	gaa ctg atg acc	ttt gga gga aaa	2790
Trp Ser Tyr Gly Val Thr Ile Trp	Glu Leu Met Thr	Phe Gly Gly Lys	
905	910	915	
ccc tat gat gga att cca acg cga	gaa atc cct gat	tta tta gag aaa	2838
Pro Tyr Asp Gly Ile Pro Thr Arg	Glu Ile Pro Asp	Leu Leu Glu Lys	
920	925	930	935
gga gaa cgt ttg cct cag cct ccc	atc tgc act att	gac gtt tac atg	2886
Gly Glu Arg Leu Pro Gln Pro Pro	Ile Cys Thr Ile	Asp Val Tyr Met	
940	945	950	
gtc atg gtc aaa tgt tgg atg att	gat gct gac agt	aga cct aaa ttt	2934
Val Met Val Lys Cys Trp Met Ile	Asp Ala Asp Ser	Arg Pro Lys Phe	
955	960	965	
aag gaa ctg gct gct gag ttt tca	agg atg gct cga	gac cct caa aga	2982
Lys Glu Leu Ala Ala Glu Phe Ser	Arg Met Ala Arg	Asp Pro Gln Arg	
970	975	980	
tac cta gtt att cag ggt gat gat	cgt atg aag ctt	ccc agt cca aat	3030
Tyr Leu Val Ile Gln Gly Asp Asp	Arg Met Lys Leu	Pro Ser Pro Asn	
985	990	995	
gac agc aag ttc ttt cag aat ctc	ttg gat gaa gag	gat ttg gaa gat	3078
Asp Ser Lys Phe Phe Gln Asn Leu	Leu Asp Glu Glu	Asp Leu Glu Asp	
1000	1005	1010	1015
atg atg gat gct gag gag tac ttg	gtc cct cag gct	ttc aac atc cca	3126
Met Met Asp Ala Glu Glu Tyr Leu	Val Pro Gln Ala	Phe Asn Ile Pro	
1020	1025	1030	
cct ccc atc tat act tcc aga gca	aga att gac tcg	aat agg agt gaa	3174
Pro Pro Ile Tyr Thr Ser Arg Ala	Arg Ile Asp Ser	Asn Arg Ser Glu	
1035	1040	1045	
att gga cac agc cct cct cct gcc	tac acc ccc atg	tca gga aac cag	3222
Ile Gly His Ser Pro Pro Pro Ala	Tyr Thr Pro Met	Ser Gly Asn Gln	
1050	1055	1060	
ttt gta tac cga gat gga ggt ttt	gct gct gaa caa	gga gtg tct gtg	3270
Phe Val Tyr Arg Asp Gly Gly Phe	Ala Ala Glu Gln	Gly Val Ser Val	
1065	1070	1075	
ccc tac aga gcc cca act agc aca	att cca gaa gct	cct gtg gca cag	3318
Pro Tyr Arg Ala Pro Thr Ser Thr	Ile Pro Glu Ala	Pro Val Ala Gln	

- 109 -

1080	1085	1090	1095	
ggc gct act gct gag att ttt gat gac tcc tgc tgt aat ggc acc cta				3366
Gly Ala Thr Val Ala Glu Ile Phe Asp Asp Ser Cys Cys Asn Gly Thr Leu				
	1100	1105	1110	
cgc aag cca gtg gca ccc cat gtc caa gag gac agt agc acc cag agg				3414
Arg Lys Pro Val Ala Pro His Val Gln Glu Asp Ser Ser Thr Gln Arg				
	1115	1120	1125	
tac agt gct gac ccc acc gtg ttt gcc cca gaa cgg agc cca cga gga				3462
Tyr Ser Ala Asp Pro Thr Val Phe Ala Pro Glu Arg Ser Pro Arg Gly				
	1130	1135	1140	
gag ctg gat gag gaa ggt tac atg act cct atg cga gac aaa ccc aaa				3510
Glu Leu Asp Glu Glu Gly Tyr Met Thr Pro Met Arg Asp Lys Pro Lys				
	1145	1150	1155	
caa gaa tac ctg aat cca gtg gag gag aac cct ttt gtt tct cgg aga				3558
Gln Glu Tyr Leu Asn Pro Val Glu Glu Asn Pro Phe Val Ser Arg Arg				
	1160	1165	1170	1175
aaa aat gga gac ctt caa gca ttg gat aat ccc gaa tat cac aat gca				3606
Lys Asn Gly Asp Leu Gln Ala Leu Asp Asn Pro Glu Tyr His Asn Ala				
	1180	1185	1190	
tcc aat ggt cca ccc aag gcc gag gat gag tat gtg aat gag cca ctg				3654
Ser Asn Gly Pro Pro Lys Ala Glu Asp Glu Tyr Val Asn Glu Pro Leu				
	1195	1200	1205	
tac ctc aac acc ttt gcc aac acc ttg gga aaa gct gag tac ctg aag				3702
Tyr Leu Asn Thr Phe Ala Asn Thr Leu Gly Lys Ala Glu Tyr Leu Lys				
	1210	1215	1220	
aac aac ata ctg tca atg cca gag aag gcc aag aaa gcg ttt gac aac				3750
Asn Asn Ile Leu Ser Met Pro Glu Lys Ala Lys Lys Ala Phe Asp Asn				
	1225	1230	1235	
cct gac tac tgg aac cac agc ctg cca cct cgg agc acc ctt cag cac				3798
Pro Asp Tyr Trp Asn His Ser Leu Pro Pro Arg Ser Thr Leu Gln His				
	1240	1245	1250	1255
cca gac tac ctg cag gag tac agc aca aaa tat ttt tat aaa cag aat				3846
Pro Asp Tyr Leu Gln Glu Tyr Ser Thr Lys Tyr Phe Tyr Lys Gln Asn				
	1260	1265	1270	
ggg cgg atc cgg cct att gtg gca gag aat cct gaa tac ctc tct gag				3894
Gly Arg Ile Arg Pro Ile Val Ala Glu Asn Pro Glu Tyr Leu Ser Glu				
	1275	1280	1285	
ttc tcc ctg aag cca ggc act gtg ctg ccg cct cca cct tac aga cac				3942
Phe Ser Leu Lys Pro Gly Thr Val Leu Pro Pro Pro Pro Tyr Arg His				

- 110 -

```

1290      1295      1300
cgg aat act gtg gtg taa gctcagttgt ggttttttag gtggagagac      3990
Arg Asn Thr Val Val
1305

acacctgctc caatttcccc acccccctct ctttctctgg tggctcttct tctaccccaa 4050
ggccagtagt tttgacactt cccagtggaa gatacagaga tgcaatgata gttatgtgct 4110
tacctaactt gaacattaga gggaaagact gaaagagaaa gataggagga accacaatgt 4170
ttcttcattt ctctgcatgg gttggtcagg agaatgaaac agctagagaa ggaccagaaa 4230
atgtaaggca atgctgccta ctatcaaaact agctgtcact ttttttcttt ttctttttct 4290
ttctttggtt ctttcttctt cttctttttt tttttttttt taaagcagat ggttgaaaca 4350
cccctgctat ctgttcctat ctgcaggaaac tgatgtgtgc atatttagca tccttggaag 4410
tcataataaa gtttccatta gaacaaaaga ataacatttt ctataacata tgatagtgtc 4470
tgaaattgag aatccagttt ctttccccag cagtttctgt cctagcaagt aagaatggcc 4530
aactcaactt tcataattta aaaatctcca ttaaagttaa aactagtaat tatgttttca 4590
acactttttg gtttttttca ttttgttttg ctctgaccga ttcttttata tttgtctccc 4650
tatttttggc tttaatttct aattgcaaag atgtttacat caaagcttct tcacagaatt 4710
taagcaagaa atattttaat atagtgaat ggccactact ttaagtatac aatcttttaa 4770
ataagaaagg gaggctaata tttttcatgc tatcaaatta tcttcaccct catcctttac 4830
atttttcaac attttttttt ctccataaat gacactactt gataggccgt tggttgtctg 4890
aagagtagaa gggaaactaa gagacagttc tctgtggttc aggaaaacta ctgatacttt 4950
caggggtggc ccaatgaggg aatccattga actggaagaa acacactgga ttgggtatgt 5010
ctacctggca gatactcaga aatgtagttt gcacttaagc tgtaatttta tttgttcttt 5070
ttctgaactc cattttggat tttgaatcaa gcaatatgga agcaaccagc aaattaacta 5130
atttaagtac attttttaaa aaagagctaa gataaagact gtggaaatgc caaaccaagc 5190
aaattaggaa ctttgcaacg gtatccaggg actatgatga gaggccagca cattatcttc 5250
atatgtcacc tttgctacgc aaggaaattt gttcagttcg tatacttcgt aagaaggaat 5310
gcgagtaagg attggcttga attccatgga atttctagta tgagactatt tatatgaagt 5370
agaaggtaac tctttgcaca taaattggta taataaaaag aaaaacacaa acattcaaag 5430
cttagggata ggtccttggg tcaaaagttg taaataaatg tgaacatct tctc      5484

```

<210> 36
 <211> 1308
 <212> PRT
 <213> Homo Sapiens

<300>
 <308> Genbank AAB59446
 <309> 1995-01-09

<400> 36
 Met Lys Pro Ala Thr Gly Leu Trp Val Trp Val Ser Leu Leu Val Ala
 1 5 10 15
 Ala Gly Thr Val Gln Pro Ser Asp Ser Gln Ser Val Cys Ala Gly Thr
 20 25 30
 Glu Asn Lys Leu Ser Ser Leu Ser Asp Leu Glu Gln Gln Tyr Arg Ala
 35 40 45
 Leu Arg Lys Tyr Tyr Glu Asn Cys Glu Val Val Met Gly Asn Leu Glu
 50 55 60
 Ile Thr Ser Ile Glu His Asn Arg Asp Leu Ser Phe Leu Arg Ser Val
 65 70 75 80

Arg	Glu	Val	Thr	Gly	Tyr	Val	Leu	Val	Ala	Leu	Asn	Gln	Phe	Arg	Tyr
				85					90					95	
Leu	Pro	Leu	Glu	Asn	Leu	Arg	Ile	Ile	Arg	Gly	Thr	Lys	Leu	Tyr	Glu
			100					105					110		
Asp	Arg	Tyr	Ala	Leu	Ala	Ile	Phe	Leu	Asn	Tyr	Arg	Lys	Asp	Gly	Asn
		115					120					125			
Phe	Gly	Leu	Gln	Glu	Leu	Gly	Leu	Lys	Asn	Leu	Thr	Glu	Ile	Leu	Asn
	130					135					140				
Gly	Gly	Val	Tyr	Val	Asp	Gln	Asn	Lys	Phe	Leu	Cys	Tyr	Ala	Asp	Thr
145					150					155					160
Ile	His	Trp	Gln	Asp	Ile	Val	Arg	Asn	Pro	Trp	Pro	Ser	Asn	Leu	Thr
				165					170					175	
Leu	Val	Ser	Thr	Asn	Gly	Ser	Ser	Gly	Cys	Gly	Arg	Cys	His	Lys	Ser
			180					185					190		
Cys	Thr	Gly	Arg	Cys	Trp	Gly	Pro	Thr	Glu	Asn	His	Cys	Gln	Thr	Leu
		195					200					205			
Thr	Arg	Thr	Val	Cys	Ala	Glu	Gln	Cys	Asp	Gly	Arg	Cys	Tyr	Gly	Pro
	210					215					220				
Tyr	Val	Ser	Asp	Cys	Cys	His	Arg	Glu	Cys	Ala	Gly	Gly	Cys	Ser	Gly
225					230					235					240
Pro	Lys	Asp	Thr	Asp	Cys	Phe	Ala	Cys	Met	Asn	Phe	Asn	Asp	Ser	Gly
				245					250					255	
Ala	Cys	Val	Thr	Gln	Cys	Pro	Gln	Thr	Phe	Val	Tyr	Asn	Pro	Thr	Thr
			260					265					270		
Phe	Gln	Leu	Glu	His	Asn	Phe	Asn	Ala	Lys	Tyr	Thr	Tyr	Gly	Ala	Phe
	275						280					285			
Cys	Val	Lys	Lys	Cys	Pro	His	Asn	Phe	Val	Val	Asp	Ser	Ser	Ser	Cys
	290					295					300				
Val	Arg	Ala	Cys	Pro	Ser	Ser	Lys	Met	Glu	Val	Glu	Glu	Asn	Gly	Ile
305					310					315					320
Lys	Met	Cys	Lys	Pro	Cys	Thr	Asp	Ile	Cys	Pro	Lys	Ala	Cys	Asp	Gly
				325					330					335	
Ile	Gly	Thr	Gly	Ser	Leu	Met	Ser	Ala	Gln	Thr	Val	Asp	Ser	Ser	Asn
			340					345					350		
Ile	Asp	Lys	Phe	Ile	Asn	Cys	Thr	Lys	Ile	Asn	Gly	Asn	Leu	Ile	Phe
	355					360						365			
Leu	Val	Thr	Gly	Ile	His	Gly	Asp	Pro	Tyr	Asn	Ala	Ile	Glu	Ala	Ile
	370					375					380				
Asp	Pro	Glu	Lys	Leu	Asn	Val	Phe	Arg	Thr	Val	Arg	Glu	Ile	Thr	Gly
385					390					395					400
Phe	Leu	Asn	Ile	Gln	Ser	Trp	Pro	Pro	Asn	Met	Thr	Asp	Phe	Ser	Val
				405					410					415	
Phe	Ser	Asn	Leu	Val	Thr	Ile	Gly	Gly	Arg	Val	Leu	Tyr	Ser	Gly	Leu
			420					425					430		
Ser	Leu	Leu	Ile	Le											

- 113 -

Leu Met Thr Phe Gly Gly Lys Pro Tyr Asp Gly Ile Pro Thr Arg Glu
 915 920 925
 Ile Pro Asp Leu Leu Glu Lys Gly Glu Arg Leu Pro Gln Pro Pro Ile
 930 935 940
 Cys Thr Ile Asp Val Tyr Met Val Met Val Lys Cys Trp Met Ile Asp
 945 950 955 960
 Ala Asp Ser Arg Pro Lys Phe Lys Glu Leu Ala Ala Glu Phe Ser Arg
 965 970 975
 Met Ala Arg Asp Pro Gln Arg Tyr Leu Val Ile Gln Gly Asp Asp Arg
 980 985 990
 Met Lys Leu Pro Ser Pro Asn Asp Ser Lys Phe Phe Gln Asn Leu Leu
 995 1000 1005
 Asp Glu Glu Asp Leu Glu Asp Met Met Asp Ala Glu Glu Tyr Leu Val
 1010 1015 1020
 Pro Gln Ala Phe Asn Ile Pro Pro Pro Ile Tyr Thr Ser Arg Ala Arg
 1025 1030 1035 1040
 Ile Asp Ser Asn Arg Ser Glu Ile Gly His Ser Pro Pro Pro Ala Tyr
 1045 1050 1055
 Thr Pro Met Ser Gly Asn Gln Phe Val Tyr Arg Asp Gly Gly Phe Ala
 1060 1065 1070
 Ala Glu Gln Gly Val Ser Val Pro Tyr Arg Ala Pro Thr Ser Thr Ile
 1075 1080 1085
 Pro Glu Ala Pro Val Ala Gln Gly Ala Thr Ala Glu Ile Phe Asp Asp
 1090 1095 1100
 Ser Cys Cys Asn Gly Thr Leu Arg Lys Pro Val Ala Pro His Val Gln
 1105 1110 1115 1120
 Glu Asp Ser Ser Thr Gln Arg Tyr Ser Ala Asp Pro Thr Val Phe Ala
 1125 1130 1135
 Pro Glu Arg Ser Pro Arg Gly Glu Leu Asp Glu Glu Gly Tyr Met Thr
 1140 1145 1150
 Pro Met Arg Asp Lys Pro Lys Gln Glu Tyr Leu Asn Pro Val Glu Glu
 1155 1160 1165
 Asn Pro Phe Val Ser Arg Arg Lys Asn Gly Asp Leu Gln Ala Leu Asp
 1170 1175 1180
 Asn Pro Glu Tyr His Asn Ala Ser Asn Gly Pro Pro Lys Ala Glu Asp
 1185 1190 1195 1200
 Glu Tyr Val Asn Glu Pro Leu Tyr Leu Asn Thr Phe Ala Asn Thr Leu
 1205 1210 1215
 Gly Lys Ala Glu Tyr Leu Lys Asn Asn Ile Leu Ser Met Pro Glu Lys
 1220 1225 1230
 Ala Lys Lys Ala Phe Asp Asn Pro Asp Tyr Trp Asn His Ser Leu Pro
 1235 1240 1245
 Pro Arg Ser Thr Leu Gln His Pro Asp Tyr Leu Gln Glu Tyr Ser Thr
 1250 1255 1260
 Lys Tyr Phe Tyr Lys Gln Asn Gly Arg Ile Arg Pro Ile Val Ala Glu
 1265 1270 1275 1280
 Asn Pro Glu Tyr Leu Ser Glu Phe Ser Leu Lys Pro Gly Thr Val Leu
 1285 1290 1295
 Pro Pro Pro Pro Tyr Arg His Arg Asn Thr Val Val
 1300 1305

- 114 -

<210> 37
 <211> 478
 <212> DNA
 <213> Mus musculus

<220>
 <221> CDS
 <222> (1)...(478)
 <223> partial nucleotide sequence encoding A387 light
 (kappa) chain

<221> variation
 <222> 15
 <223> n is any

<400> 37
 gay aty gts cts acn cag wsb ccb gcc acc ctg tct gtg tct cca gga
 48
 Asp Xaa Xaa Xaa Thr Gln Xaa Xaa Ala Thr Leu Ser Val Ser Pro Gly
 1 5 10 15
 gat agc gtc agt ctt tcc tgc agg gcc agc caa agt atc agc aac aac
 96
 Asp Ser Val Ser Leu Ser Cys Arg Ala Ser Gln Ser Ile Ser Asn Asn
 20 25 30
 cta cac tgg tat caa caa aaa tca cat gag tct cca agg att ctc atc
 144
 Leu His Trp Tyr Gln Gln Lys Ser His Glu Ser Pro Arg Ile Leu Ile
 35 40 45
 aag tat gca tcc cag tcc atc tat ggg atc ccc tca agg ttc agt ggc
 192
 Lys Tyr Ala Ser Gln Ser Ile Tyr Gly Ile Pro Ser Arg Phe Ser Gly
 50 55 60
 agt gga tca ggg aca ttt ttc act ctc att gtc aac agt gtg ggg act
 240
 Ser Gly Ser Gly Thr Phe Phe Thr Leu Ile Val Asn Ser Val Gly Thr
 65 70 75 80
 gaa gat ttt gga atg tat ttc tgt caa cag agt cac agc tgg cct ctc
 288
 Glu Asp Phe Gly Met Tyr Phe Cys Gln Gln Ser His Ser Trp Pro Leu
 85 90 95
 acg ttc ggt act ggg acc aag ctg gag ctg aaa cgg gct gat gct gca
 336
 Thr Phe Gly Thr Gly Thr Lys Leu Glu Leu Lys Arg Ala Asp Ala Ala
 100 105 110

- 115 -

cca act gta tcc atc ttc cca cca tcc agt gag cag tta aca tct gga
384

Pro Thr Val Ser Ile Phe Pro Pro Ser Ser Glu Gln Leu Thr Ser Gly
115 120 125

ggc gcc tca gtc gtg tgc ttc ttg aac aac ttc tac ccc aga gac atc
432

Gly Ala Ser Val Val Cys Phe Leu Asn Asn Phe Tyr Pro Arg Asp Ile
130 135 140

aat gtc aag tgg aag att gat ggc agt gaa cga caa aat ggc gtc c
478

Asn Val Lys Trp Lys Ile Asp Gly Ser Glu Arg Gln Asn Gly Val
145 150 155

<210> 38

<211> 159

<212> PRT

<213> Mus musculus

<220>

<221> VARIANT

<222> 2

<223> Xaa is Ile

<221> VARIANT

<222> 3

<223> Xaa is Val

<221> VARIANT

<222> 4

<223> Xaa is Leu

<221> VARIANT

<222> 5

<223> Xaa is Thr

<221> VARIANT

<222> 7

<223> Xaa is Thr, Arg, Ser, Cys or Trp

<400> 38

Asp Xaa Xaa Xaa Thr Gln Xaa Xaa Ala Thr Leu Ser Val Ser Pro Gly
1 5 10 15

Asp Ser Val Ser Leu Ser Cys Arg Ala Ser Gln Ser Ile Ser Asn Asn
20 25 30

Leu His Trp Tyr Gln Gln Lys Ser His Glu Ser Pro Arg Ile Leu Ile
35 40 45

Lys Tyr Ala Ser Gln Ser Ile Tyr Gly Ile Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Phe Phe Thr Leu Ile Val Asn Ser Val Gly Thr

- 116 -

```

65              70              75              80
Glu Asp Phe Gly Met Tyr Phe Cys Gln Gln Ser His Ser Trp Pro Leu
              85              90              95
Thr Phe Gly Thr Gly Thr Lys Leu Glu Leu Lys Arg Ala Asp Ala Ala
              100             105             110
Pro Thr Val Ser Ile Phe Pro Pro Ser Ser Glu Gln Leu Thr Ser Gly
              115             120             125
Gly Ala Ser Val Val Cys Phe Leu Asn Asn Phe Tyr Pro Arg Asp Ile
              130             135             140
Asn Val Lys Trp Lys Ile Asp Gly Ser Glu Arg Gln Asn Gly Val
145              150              155

```

```

<210> 39
<211> 366
<212> DNA
<213> Mus musculus

```

```

<220>
<221> CDS
<222> (1)...(366)
<223> partial nucleotide sequence encoding A387 heavy
      (igG2a) chain sequence

```

```

<400> 39
gag gtt aag ytk gtt gar tct gga gga gac tta gtg aaa cct gga ggg
48
Glu Val Lys Xaa Val Glu Ser Gly Gly Asp Leu Val Lys Pro Gly Gly
 1              5              10              15

tcc ctg aaa ctc gcc tgt gca gcc tct gga ttc act ttc agt aac gat
96
Ser Leu Lys Leu Ala Cys Ala Ala Ser Gly Phe Thr Phe Ser Asn Asp
              20              25              30

gcc atg tct tgg gtt cgc cag act cca gaa aag agg ctg gag tgg gtc
144
Ala Met Ser Trp Val Arg Gln Thr Pro Glu Lys Arg Leu Glu Trp Val
              35              40              45

gca tcc att agt agt gtt ggt aac acc tac tat cca gac agt gtg aag
192
Ala Ser Ile Ser Ser Val Gly Asn Thr Tyr Tyr Pro Asp Ser Val Lys
              50              55              60

ggc cga ttc acc atc tcc aga gat aat gcc agg aac att cta tac ctg
240
Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Arg Asn Ile Leu Tyr Leu
65              70              75              80

caa atg agt agt gtg agg tct gag gac acg gcc atg tat tac tgt gca
288
Gln Met Ser Ser Val Arg Ser Glu Asp Thr Ala Met Tyr Tyr Cys Ala

```

- 117 -

85 90 95
 aga ggc tat ggt gtt agt ccc tgg ttt tct tac tgg ggc caa ggg act
 336
 Arg Gly Tyr Gly Val Ser Pro Trp Phe Ser Tyr Trp Gly Gln Gly Thr
 100 105 110

cta gtc acc gtc tcc tca gcc aaa aca aca
 366
 Leu Val Thr Val Ser Ser Ala Lys Thr Thr
 115 120

<210> 40
 <211> 122
 <212> PRT
 <213> Mus musculus

<220>
 <221> VARIANT
 <222> 4
 <223> Xaa is Leu or Phe

<400> 40
 Glu Val Lys Xaa Val Glu Ser Gly Gly Asp Leu Val Lys Pro Gly Gly
 1 5 10 15
 Ser Leu Lys Leu Ala Cys Ala Ala Ser Gly Phe Thr Phe Ser Asn Asp
 20 25 30
 Ala Met Ser Trp Val Arg Gln Thr Pro Glu Lys Arg Leu Glu Trp Val
 35 40 45
 Ala Ser Ile Ser Ser Val Gly Asn Thr Tyr Tyr Pro Asp Ser Val Lys
 50 55 60
 Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Arg Asn Ile Leu Tyr Leu
 65 70 75 80
 Gln Met Ser Ser Val Arg Ser Glu Asp Thr Ala Met Tyr Tyr Cys Ala
 85 90 95
 Arg Gly Tyr Gly Val Ser Pro Trp Phe Ser Tyr Trp Gly Gln Gly Thr
 100 105 110
 Leu Val Thr Val Ser Ser Ala Lys Thr Thr
 115 120

<210> 41
 <211> 493
 <212> DNA
 <213> Mus musculus

<220>
 <221> CDS
 <222> (1)...(493)
 <223> partial nucleotide sequence encoding B436 (kappa)
 Light Chain V-J region

- 118 -

<400> 41

gay gty ytb atg acy car acy cca ctc tcc ctg cct gtc agt ctt gga
48

Asp Xaa Xaa Met Xaa Gln Xaa Pro Leu Ser Leu Pro Val Ser Leu Gly
1 5 10 15

gat caa gcc tcc atc tct tgc aga tct agt cag aac att gta cat agt
96

Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Asn Ile Val His Ser
20 25 30

agt gga aac acc tat tta gaa tgg tac ctg cag aaa cca ggc cag tct
144

Ser Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45

cca aag ctc ctg atc tac aaa gtt tcc aac cga ttt tct ggg gtc cca
192

Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60

gac agg ttc agt ggc agt gga tca ggg aca gat ttc aca ctc aag atc
240

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

agc aga gtg gag gct gag gat ctg gga att tat tac tgc ttt caa ggt
288

Ser Arg Val Glu Ala Glu Asp Leu Gly Ile Tyr Tyr Cys Phe Gln Gly
85 90 95

tca cat gtt ccg tac acg ttc gga ggg ggg acc aag ctg gaa ata aaa
336

Ser His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105 110

cgg gct gat gct gca cca act gta tcc atc ttc cca cca tcc agt gag
384

Arg Ala Asp Ala Ala Pro Thr Val Ser Ile Phe Pro Pro Ser Ser Glu
115 120 125

cag tta aca tct gga ggt gcc tca gtc gtg tgc ttc ttg aac aac ttc
432

Gln Leu Thr Ser Gly Gly Ala Ser Val Val Cys Phe Leu Asn Asn Phe
130 135 140

tac ccc aga gac atc aat gtc aag tgg aag att gat ggc agt gaa cga
480

Tyr Pro Arg Asp Ile Asn Val Lys Trp Lys Ile Asp Gly Ser Glu Arg
145 150 155 160

- 119 -

caa aat ggc gtc c
493
Gln Asn Gly Val

<210> 42
<211> 164
<212> PRT
<213> Mus musculus

<220>
<221> VARIANT
<222> 2
<223> Xaa is Val

<221> VARIANT
<222> 3
<223> Xaa is Phe or Leu

<221> VARIANT
<222> 5
<223> Xaa is Thr

<221> VARIANT
<222> 7
<223> Xaa is Thr

<400> 42
Asp Xaa Xaa Met Xaa Gln Xaa Pro Leu Ser Leu Pro Val Ser Leu Gly
1 5 10 15
Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Asn Ile Val His Ser
20 25 30
Ser Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45
Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80
Ser Arg Val Glu Ala Glu Asp Leu Gly Ile Tyr Tyr Cys Phe Gln Gly
85 90 95
Ser His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105 110
Arg Ala Asp Ala Ala Pro Thr Val Ser Ile Phe Pro Pro Ser Ser Glu
115 120 125
Gln Leu Thr Ser Gly Gly Ala Ser Val Val Cys Phe Leu Asn Asn Phe
130 135 140
Tyr Pro Arg Asp Ile Asn Val Lys Trp Lys Ile Asp Gly Ser Glu Arg
145 150 155 160
Gln Asn Gly Val

- 120 -

<210> 43
 <211> 354
 <212> DNA
 <213> Mus musculus

<220>
 <221> CDS
 <222> (1)...(354)
 <223> partial nucleotide sequence encoding B436 heavy
 (IgG2a) chain

<400> 43
 gag gty atg ytk gty gar tct gga gga ggc tta gtg aag cct gga ggg
 48
 Glu Xaa Met Xaa Xaa Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
 1 5 10 15
 tcc ctg aaa ctc tcc tgt gta gcc tct gga ttc act ttc agt aga tat
 96
 Ser Leu Lys Leu Ser Cys Val Ala Ser Gly Phe Thr Phe Ser Arg Tyr
 20 25 30
 acc atg tct tgg gtt cgc cag act ccg gcg aag aga ctg gag tgg gtc
 144
 Thr Met Ser Trp Val Arg Gln Thr Pro Ala Lys Arg Leu Glu Trp Val
 35 40 45
 gca acc atc aat ttt ggt aat ggt aac acc tac tat cct gac agt gtg
 192
 Ala Thr Ile Asn Phe Gly Asn Gly Asn Thr Tyr Tyr Pro Asp Ser Val
 50 55 60
 aag ggc cga ttc acc atc tcc aga gac aat gcc agg aac acc ctg tat
 240
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Arg Asn Thr Leu Tyr
 65 70 75 80
 ctg caa atg agc agt ctg agg tct gag gac acg gcc atg tat tac tgt
 288
 Leu Gln Met Ser Ser Leu Arg Ser Glu Asp Thr Ala Met Tyr Tyr Cys
 85 90 95
 aca agc ctt aat tgg gct tac tgg ggc caa ggg act ctg gtc acc gtc
 336
 Thr Ser Leu Asn Trp Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr Val
 100 105 110
 tcc tca gcc aaa aca aca
 354
 Ser Ser Ala Lys Thr Thr
 115

- 121 -

<210> 44
 <211> 118
 <212> PRT
 <213> Mus musculus

<220>
 <221> VARIANT
 <222> 2
 <223> Xaa is Val

<221> VARIANT
 <222> 4
 <223> Xaa is Phe or Leu

<221> VARIANT
 <222> 5
 <223> Xaa is Val

<400> 44
 Glu Xaa Met Xaa Xaa Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
 1 5 10 15
 Ser Leu Lys Leu Ser Cys Val Ala Ser Gly Phe Thr Phe Ser Arg Tyr
 20 25 30
 Thr Met Ser Trp Val Arg Gln Thr Pro Ala Lys Arg Leu Glu Trp Val
 35 40 45
 Ala Thr Ile Asn Phe Gly Asn Gly Asn Thr Tyr Tyr Pro Asp Ser Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Arg Asn Thr Leu Tyr
 65 70 75 80
 Leu Gln Met Ser Ser Leu Arg Ser Glu Asp Thr Ala Met Tyr Tyr Cys
 85 90 95
 Thr Ser Leu Asn Trp Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr Val
 100 105 110
 Ser Ser Ala Lys Thr Thr
 115

<210> 45
 <211> 36
 <212> DNA
 <213> Mus musculus

<220>
 <221> CDS
 <222> (1)...(36)
 <223> Mouse immunoglobulin Light Chain J segment

<300>
 <308> GenBank #z66550
 <309> 1997-12-15

<400> 45

- 122 -

tac acg ttc gga ggg ggg acc aag ctg gaa ata aaa
36
Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
1 5 10

<210> 46
<211> 12
<212> PRT
<213> Mus musculus

<400> 46
Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
1 5 10

<210> 47
<211> 21
<212> DNA
<213> Mus musculus

<220>
<221> CDS
<222> (1)...(21)
<223> Mouse immunoglobulin Light Chain V-J region

<300>
<308> GenBank #Z66550
<309> 1997-12-15

<400> 47
cta cag tat gat gag ctt cca
21
Leu Gln Tyr Asp Glu Leu Pro
1 5

<210> 48
<211> 7
<212> PRT
<213> Mus musculus

<400> 48.
Leu Gln Tyr Asp Glu Leu Pro
1 5

<210> 49
<211> 34
<212> DNA
<213> Mus musculus

<220>
<221> CDS

- 123 -

<222> (1)...(34)
<223> Mouse Immunoglobulin Light Chain J region

<300>
<308> GenBank #Z31353
<309> 1995-09-27

<400> 49
gac gtt cgg tgg agg cac caa gct gga aat caa a
34
Asp Val Arg Trp Arg His Gln Ala Gly Asn Gln
1 5 10

<210> 50
<211> 11
<212> PRT
<213> Mus musculus

<400> 50
Asp Val Arg Trp Arg His Gln Ala Gly Asn Gln
1 5 10

<210> 51
<211> 39
<212> DNA
<213> Mus musculus

<220>
<221> CDS
<222> (1)...(39)
<223> Mouse Immunoglobulin Light Chain J region

<300>
<308> GenBank #X87231
<309> 1995-06-06

<400> 51
gtg gac gtt cgg tgg agg cac caa gct gga aat caa acg
39
Val Asp Val Arg Trp Arg His Gln Ala Gly Asn Gln Thr
1 5 10

<210> 52
<211> 13
<212> PRT
<213> Mus musculus

<400> 52
Val Asp Val Arg Trp Arg His Gln Ala Gly Asn Gln Thr
1 5 10

- 124 -

<210> 53
<211> 33
<212> DNA
<213> Mus musculus

<220>
<221> CDS
<222> (1)...(33)
<223> mouse immunoglobulin light chain J region

<300>
<308> GenBank #L21025
<309> 1994-10-29

<400> 53
tgg acg ttc ggt gga ggc acc aag ctg gaa atc
33
Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile
1 5 10

<210> 54
<211> 11
<212> PRT
<213> Mus musculus

<400> 54
Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile
1 5 10

<210> 55
<211> 12
<212> PRT
<213> Mus musculus

<220>
<221> DOMAIN
<222> (1)...(12)
<223> Mouse immunoglobulin light chain J-region

<300>
<308> GenBank #Z6650
<309> 1997-12-15

<400> 55
Arg Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
1 5 10

<210> 56
<211> 36
<212> DNA

- 125 -

<213> Mus musculus

<220>

<221> CDS

<222> (1)...(36)

<223> Mouse immunoglobulin light chain J region

<300>

<308> GenBank #L21021

<309> 1994-10-29

<400> 56

ctg acg ttc ggt gga ggc acc aag ctg gaa atc aat
36

Leu Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Asn
1 5 10

<210> 57

<211> 12

<212> PRT

<213> Mus musculus

<400> 57

Leu Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Asn
1 5 10

<210> 58

<211> 36

<212> DNA

<213> Mus musculus

<220>

<221> CDS

<222> (1)...(36)

<223> mouse immunoglobulin light chain J region

<300>

<308> GenBank #L21019

<309> 1994-10-29

<400> 58

ccg acg ttc ggt gga ggc acc aag ctg gaa atc acc
36

Pro Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Thr
1 5 10

<210> 59

<211> 12

<212> PRT

<213> Mus musculus

- 126 -

<400> 59
Pro Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Thr
1 5 10

<210> 60
<211> 36
<212> DNA
<213> Mus musculus

<220>
<221> CDS
<222> (1)...(36)
<223> mouse immunoglobulin light chain J region

<300>
<308> GenBank #L21017
<309> 1994-10-29

<400> 60
ccg acg ttc ggt gga ggc acc aag ctg gaa atc aaa
36
Pro Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
1 5 10

<210> 61
<211> 12
<212> PRT
<213> Mus musculus

<400> 61
Pro Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
1 5 10

<210> 62
<211> 319
<212> DNA
<213> Mus musculus

<220>
<221> CDS
<222> (1)...(319)
<223> mouse immunoglobulin light chain Constant region

<300>
<308> GenBank #X87231
<309> 1995-06-06

<400> 62

- 127 -

ggc tga tgc tgc acc aac tgt atc cat ctt ccc acc atc cag tga gca
48

Gly Cys Cys Thr Asn Cys Ile His Leu Pro Thr Ile Gln Ala
1 5 10

gtt aac atc tgg agg tgc ctc agt cgt gtg ctt ctt gaa caa ctt cta
96

Val Asn Ile Trp Arg Cys Leu Ser Arg Val Leu Leu Glu Gln Leu Leu
15 20 25 30

ccc caa aga cat caa tgt caa gtg gaa gat tga tgg cag tga acg aca
144

Pro Gln Arg His Gln Cys Gln Val Glu Asp Trp Gln Thr Thr
35 40

aaa tgg cgt cct gaa cag ttg gac tga tca gga cag caa aga cag cac
192

Lys Trp Arg Pro Glu Gln Leu Asp Ser Gly Gln Gln Arg Gln His
45 50 55

cta cag cat gag cag cac cct cac gtt gac caa gga cga gta tga acg
240

Leu Gln His Glu Gln His Pro His Val Asp Gln Gly Arg Val Thr
60 65 70

aca taa cag cta tac ctg tga ggc cac tca caa gac atc tac ttc acc
288

Thr Gln Leu Tyr Leu Gly His Ser Gln Asp Ile Tyr Phe Thr
75 80 85

cat tgt caa gag ctt caa cag gaa tga gtg t

319

His Cys Gln Glu Leu Gln Gln Glu Val
90 95

<210> 63

<211> 97

<212> PRT

<213> Mus musculus

<400> 63

Gly Cys Cys Thr Asn Cys Ile His Leu Pro Thr Ile Gln Ala Val Asn
1 5 10 15
Ile Trp Arg Cys Leu Ser Arg Val Leu Glu Gln Leu Leu Pro Gln
20 25 30
Arg His Gln Cys Gln Val Glu Asp Trp Gln Thr Thr Lys Trp Arg Pro
35 40 45
Glu Gln Leu Asp Ser Gly Gln Gln Arg Gln His Leu Gln His Glu Gln
50 55 60
His Pro His Val Asp Gln Gly Arg Val Thr Thr Gln Leu Tyr Leu Gly
65 70 75 80

- 128 -

His Ser Gln Asp Ile Tyr Phe Thr His Cys Gln Glu Leu Gln Gln Glu
 85 90 95
 Val

<210> 64
 <211> 321
 <212> DNA
 <213> Mus musculus

<220>
 <221> CDS
 <222> (1)...(321)
 <223> mouse partial nucleotide sequence for
 immunoglobulin light chain Constant region

<300>
 <308> GenBank #AJ294736
 <309> 2001-02-09

<400> 64
 gct gat gct gca cca act gta tcc atc ttc cca cca tcc agt gag cag
 48
 Ala Asp Ala Ala Pro Thr Val Ser Ile Phe Pro Pro Ser Ser Glu Gln
 1 5 10 15
 tta aca tct gga ggt gcc tca gtc gtg tgc ttc ttg aac aac ttc tac
 96
 Leu Thr Ser Gly Gly Ala Ser Val Val Cys Phe Leu Asn Asn Phe Tyr
 20 25 30
 ccc aaa gac atc aat gtc aag tgg aag att gat ggc agt gaa cga caa
 144
 Pro Lys Asp Ile Asn Val Lys Trp Lys Ile Asp Gly Ser Glu Arg Gln
 35 40 45
 aat ggc gtc ctg aac agt tgg act gat cag gac agc aaa gac agc acc
 192
 Asn Gly Val Leu Asn Ser Trp Thr Asp Gln Asp Ser Lys Asp Ser Thr
 50 55 60
 tac agc atg agc agc acc ctc acg ttg acc aag gac gag tat gaa cga
 240
 Tyr Ser Met Ser Ser Thr Leu Thr Leu Thr Lys Asp Glu Tyr Glu Arg
 65 70 75 80
 cat aac agc tat acc tgt gag gcc act cac aag aca tca act tca ccc
 288
 His Asn Ser Tyr Thr Cys Glu Ala Thr His Lys Thr Ser Thr Ser Pro
 85 90 95

- 129 -

atc gtc aag agc ttc aac agg aat gag tgt tag
 321
 Ile Val Lys Ser Phe Asn Arg Asn Glu Cys
 100 105

<210> 65
 <211> 106
 <212> PRT
 <213> Mus musculus

<400> 65
 Ala Asp Ala Ala Pro Thr Val Ser Ile Phe Pro Pro Ser Ser Glu Gln
 1 5 10 15
 Leu Thr Ser Gly Gly Ala Ser Val Val Cys Phe Leu Asn Asn Phe Tyr
 20 25 30
 Pro Lys Asp Ile Asn Val Lys Trp Lys Ile Asp Gly Ser Glu Arg Gln
 35 40 45
 Asn Gly Val Leu Asn Ser Trp Thr Asp Gln Asp Ser Lys Asp Ser Thr
 50 55 60
 Tyr Ser Met Ser Ser Thr Leu Thr Leu Thr Lys Asp Glu Tyr Glu Arg
 65 70 75 80
 His Asn Ser Tyr Thr Cys Glu Ala Thr His Lys Thr Ser Thr Ser Pro
 85 90 95
 Ile Val Lys Ser Phe Asn Arg Asn Glu Cys
 100 105

<210> 66
 <211> 48
 <212> DNA
 <213> Mus musculus

<220>
 <221> CDS
 <222> (1)...(48)
 <223> mouse antibody heavy chain J region

<300>
 <308> GenBank #Z66553
 <309> 1997-12-15

<400> 66
 tgg aac ttc gat gtc tgg ggc gca ggg acc acg gtc acc gtc tcc tca
 48
 Trp Asn Phe Asp Val Trp Gly Ala Gly Thr Thr Val Thr Val Ser Ser
 1 5 10 15

<210> 67
 <211> 15
 <212> PRT
 <213> Mus musculus

- 130 -

<400> 67

Asn Phe Asp Val Trp Gly Ala Gly Thr Thr Val Thr Val Ser Ser
 1 5 10 15

<210> 68

<211> 990

<212> DNA

<213> Mus musculus

<220>

<221> CDS

<222> (1)...(991)

<223> mouse immunoglobulin heavy chain Constant region

<300>

<308> GenBank #AJ294738

<309> 2001-02-09

<400> 68

gcc aaa aca aca gcc cca tcg gtc tat cca ctg gcc cct gtg tgt gga
 48

Ala Lys Thr Thr Ala Pro Ser Val Tyr Pro Leu Ala Pro Val Cys Gly
 1 5 10 15

gat aca act ggc tcc tcg gtg act cta gga tgc ctg gtc aag ggt tat
 96

Asp Thr Thr Gly Ser Ser Val Thr Leu Gly Cys Leu Val Lys Gly Tyr
 20 25 30

ttc cct gag cca gtg acc ttg acc tgg aac tct gga tcc ctg tcc agt
 144

Phe Pro Glu Pro Val Thr Leu Thr Trp Asn Ser Gly Ser Leu Ser Ser
 35 40 45

ggg gtg cac acc ttc cca gct gtc ctg cag tct gac ctc tac acc ctc
 192

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Asp Leu Tyr Thr Leu
 50 55 60

agc agc tca gtg act gta acc tcg agc acc tgg ccc agc cag tcc atc
 240

Ser Ser Ser Val Thr Val Thr Ser Ser Thr Trp Pro Ser Gln Ser Ile
 65 70 75 80

acc tgc aat gtg gcc cac ccg gca agc agc acc aag gtg gac aag aaa
 288

Thr Cys Asn Val Ala His Pro Ala Ser Ser Thr Lys Val Asp Lys Lys
 85 90 95

att gag ccc aga ggg ccc aca atc aag ccc tgt cct cca tgc aaa tgc
 336

- 131 -

Ile Glu Pro Arg Gly Pro Thr Ile Lys Pro Cys Pro Pro Cys Lys Cys
 100 105 110
 cca gca cct aac ctc ttg ggt gga cca tcc gtc ttc atc ttc cct cca
 384
 Pro Ala Pro Asn Leu Leu Gly Gly Pro Ser Val Phe Ile Phe Pro Pro
 115 120 125
 aag atc aag gat gta ctc atg atc tcc ctg agc ccc ata gtc aca tgt
 432
 Lys Ile Lys Asp Val Leu Met Ile Ser Leu Ser Pro Ile Val Thr Cys
 130 135 140
 gtg gtg gtg gat gtg agc gag gat gac cca gat gtc cag atc agc tgg
 480
 Val Val Val Asp Val Ser Glu Asp Asp Pro Asp Val Gln Ile Ser Trp
 145 150 155 160
 ttt gtg aac aac gtg gaa gta cac aca gct cag aca caa acc cat aga
 528
 Phe Val Asn Asn Val Glu Val His Thr Ala Gln Thr Gln Thr His Arg
 165 170 175
 gag gat tac aac agt act ctc cgg gtg gtc agt gcc ctc ccc atc cag
 576
 Glu Asp Tyr Asn Ser Thr Leu Arg Val Val Ser Ala Leu Pro Ile Gln
 180 185 190
 cac cag gac tgg atg agt ggc aag gag ttc aaa tgc aag gtc aac aac
 624
 His Gln Asp Trp Met Ser Gly Lys Glu Phe Lys Cys Lys Val Asn Asn
 195 200 205
 aaa gac ctc cca gcg ccc atc gag aga acc atc tca aaa ccc aaa ggg
 672
 Lys Asp Leu Pro Ala Pro Ile Glu Arg Thr Ile Ser Lys Pro Lys Gly
 210 215 220
 tca gta aga gct cca cag gta tat gtc ttg cct cca cca gaa gaa gag
 720
 Ser Val Arg Ala Pro Gln Val Tyr Val Leu Pro Pro Pro Glu Glu Glu
 225 230 235 240
 atg act aag aaa cag gtc act ctg acc tgc atg gtc aca gac ttc atg
 768
 Met Thr Lys Lys Gln Val Thr Leu Thr Cys Met Val Thr Asp Phe Met
 245 250 255
 cct gaa gac att tac gtg gag tgg acc aac aac ggg aaa aca gag cta
 816
 Pro Glu Asp Ile Tyr Val Glu Trp Thr Asn Asn Gly Lys Thr Glu Leu
 260 265 270

- 132 -

aac tac aag aac act gaa cca gtc ctg gac tct gat ggt tct tac ttc
864
Asn Tyr Lys Asn Thr Glu Pro Val Leu Asp Ser Asp Gly Ser Tyr Phe
275 280 285

atg tac agc aag ctg aga gtg gaa aag aag aac tgg gtg gaa aga aat
912
Met Tyr Ser Lys Leu Arg Val Glu Lys Lys Asn Trp Val Glu Arg Asn
290 295 300

agc tac tcc tgt tca gtg gtc cac gag ggt ctg cac aat cac cac acg
960
Ser Tyr Ser Cys Ser Val Val His Glu Gly Leu His Asn His His Thr
305 310 315 320

act aag agc ttc tcc cgg act ccg ggt aaa
990
Thr Lys Ser Phe Ser Arg Thr Pro Gly Lys
325 330

<210> 69
<211> 329
<212> PRT
<213> Mus musculus

<400> 69
Lys Thr Thr Ala Pro Ser Val Tyr Pro Leu Ala Pro Val Cys Gly Asp
1 5 10 15
Thr Thr Gly Ser Ser Val Thr Leu Gly Cys Leu Val Lys Gly Tyr Phe
20 25 30
Pro Glu Pro Val Thr Leu Thr Trp Asn Ser Gly Ser Leu Ser Ser Gly
35 40 45
Val His Thr Phe Pro Ala Val Leu Gln Ser Asp Leu Tyr Thr Leu Ser
50 55 60
Ser Ser Val Thr Val Thr Ser Ser Thr Trp Pro Ser Gln Ser Ile Thr
65 70 75 80
Cys Asn Val Ala His Pro Ala Ser Ser Thr Lys Val Asp Lys Lys Ile
85 90 95
Glu Pro Arg Gly Pro Thr Ile Lys Pro Cys Pro Pro Cys Lys Cys Pro
100 105 110
Ala Pro Asn Leu Leu Gly Gly Pro Ser Val Phe Ile Phe Pro Pro Lys
115 120 125
Ile Lys Asp Val Leu Met Ile Ser Leu Ser Pro Ile Val Thr Cys Val
130 135 140
Val Val Asp Val Ser Glu Asp Asp Pro Asp Val Gln Ile Ser Trp Phe
145 150 155 160
Val Asn Asn Val Glu Val His Thr Ala Gln Thr Gln Thr His Arg Glu
165 170 175
Asp Tyr Asn Ser Thr Leu Arg Val Val Ser Ala Leu Pro Ile Gln His
180 185 190

- 133 -

Gln Asp Trp Met Ser Gly Lys Glu Phe Lys Cys Lys Val Asn Asn Lys
 195 200 205
 Asp Leu Pro Ala Pro Ile Glu Arg Thr Ile Ser Lys Pro Lys Gly Ser
 210 215 220
 Val Arg Ala Pro Gln Val Tyr Val Leu Pro Pro Pro Glu Glu Glu Met
 225 230 235 240
 Thr Lys Lys Gln Val Thr Leu Thr Cys Met Val Thr Asp Phe Met Pro
 245 250 255
 Glu Asp Ile Tyr Val Glu Trp Thr Asn Asn Gly Lys Thr Glu Leu Asn
 260 265 270
 Tyr Lys Asn Thr Glu Pro Val Leu Asp Ser Asp Gly Ser Tyr Phe Met
 275 280 285
 Tyr Ser Lys Leu Arg Val Glu Lys Lys Asn Trp Val Glu Arg Asn Ser
 290 295 300
 Tyr Ser Cys Ser Val Val His Glu Gly Leu His Asn His His Thr Thr
 305 310 315 320
 Lys Ser Phe Ser Arg Thr Pro Gly Lys
 325

<210> 70

<211> 975

<212> DNA

<213> Mus musculus

<220>

<221> CDS

<222> (1)..(972)

<223> mouse immunoglobulin heavy chain Constant region

<300>

<308> GenBank #M60435

<309> 2001-05-16

<400> 70

gcc aaa acg aca ccc cca tct gtc tat cca ctg gcc cct gga tct gct
48

Ala Lys Thr Thr Pro Pro Ser Val Tyr Pro Leu Ala Pro Gly Ser Ala
1 5 10 15

gcc caa act aac tcc atg gtg acc ctg gga tgc ctg gtc aag ggc tat
96

Ala Gln Thr Asn Ser Met Val Thr Leu Gly Cys Leu Val Lys Gly Tyr
20 25 30

ttc cct gag cca gtg aca gtg acc tgg aac tct gga tcc ctg tcc agc
144

Phe Pro Glu Pro Val Thr Val Thr Trp Asn Ser Gly Ser Leu Ser Ser
35 40 45

ggt gtg cac acc ttc cca gct gtc ctg cag tct gac ctc tac act ctg
192

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Asp Leu Tyr Thr Leu

- 134 -

```

50              55              60
agc agc tca gtg act gtc ccc tcc agc acc tgg ccc agc gag acc gtc
240
Ser Ser Ser Val Thr Val Pro Ser Ser Thr Trp Pro Ser Glu Thr Val
65              70              75              80

acc tgc aac gtt gcc cac ccg gcc agc agc acc aag gtg gac aag aaa
288
Thr Cys Asn Val Ala His Pro Ala Ser Ser Thr Lys Val Asp Lys Lys
85              90              95

att gtg ccc agg gat tgt ggt tgt aag cct tgc ata tgt aca gtc cca
336
Ile Val Pro Arg Asp Cys Gly Cys Lys Pro Cys Ile Cys Thr Val Pro
100              105              110

gaa gta tca tct gtc ttc atc ttc ccc cca aag ccc aag gat gtg ctc
384
Glu Val Ser Ser Val Phe Ile Phe Pro Pro Lys Pro Lys Asp Val Leu
115              120              125

acc att act ctg act cct aag gtc acg tgt gtt gtg gta gac atc agc
432
Thr Ile Thr Leu Thr Pro Lys Val Thr Cys Val Val Val Asp Ile Ser
130              135              140

aag gat gat ccc gag gtc cag ttc agc tgg ttt gta gat gat gtg gag
480
Lys Asp Asp Pro Glu Val Gln Phe Ser Trp Phe Val Asp Asp Val Glu
145              150              155              160

gtg cac aca gct cag acg caa ccc cgg gag gag cag ttc aac agc act
528
Val His Thr Ala Gln Thr Gln Pro Arg Glu Glu Gln Phe Asn Ser Thr
165              170              175

ttc cgc tca gtc agt gaa ctt ccc atc atg cac cag gac tgg ctc aat
576
Phe Arg Ser Val Ser Glu Leu Pro Ile Met His Gln Asp Trp Leu Asn
180              185              190

ggc aag gag ttc aaa tgc agg gtc aac agt gca gct ttc cct gcc ccc
624
Gly Lys Glu Phe Lys Cys Arg Val Asn Ser Ala Ala Phe Pro Ala Pro
195              200              205

atc gag aaa acc atc tcc aaa acc aaa ggc aga ccg aag gct cca cag
672
Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly Arg Pro Lys Ala Pro Gln
210              215              220

```

- 135 -

gtg tac acc att cca cct ccc aag gag cag atg gcc aag gat aaa gtc
720

Val Tyr Thr Ile Pro Pro Pro Lys Glu Gln Met Ala Lys Asp Lys Val
225 230 235 240

agt ctg acc tgc atg ata aca gac ttc ttc cct gaa gac att act gtg
768

Ser Leu Thr Cys Met Ile Thr Asp Phe Phe Pro Glu Asp Ile Thr Val
245 250 255

gag tgg cag tgg aat ggg cag cca gcg gag aac tac aag aac act cag
816

Glu Trp Gln Trp Asn Gly Gln Pro Ala Glu Asn Tyr Lys Asn Thr Gln
260 265 270

ccc atc atg gac aca gat ggc tct tac ttc gtc tac agc aag ctc aat
864

Pro Ile Met Asp Thr Asp Gly Ser Tyr Phe Val Tyr Ser Lys Leu Asn
275 280 285

gtg cag aag agc aac tgg gag gca gga aat act ttc acc tgc tct gtg
912

Val Gln Lys Ser Asn Trp Glu Ala Gly Asn Thr Phe Thr Cys Ser Val
290 295 300

tta cat gag ggc ctg cac aac cac cat act gag aag agc ctc tcc cac
960

Leu His Glu Gly Leu His Asn His His Thr Glu Lys Ser Leu Ser His
305 310 315 320

tct cct ggt aaa tga

975

Ser Pro Gly Lys

<210> 71

<211> 324

<212> PRT

<213> Mus musculus

<400> 71

Ala Lys Thr Thr Pro Pro Ser Val Tyr Pro Leu Ala Pro Gly Ser Ala
1 5 10 15

Ala Gln Thr Asn Ser Met Val Thr Leu Gly Cys Leu Val Lys Gly Tyr
20 25 30

Phe Pro Glu Pro Val Thr Val Thr Trp Asn Ser Gly Ser Leu Ser Ser
35 40 45

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Asp Leu Tyr Thr Leu
50 55 60

Ser Ser Ser Val Thr Val Pro Ser Ser Thr Trp Pro Ser Glu Thr Val
65 70 75 80

- 136 -

```

Thr Cys Asn Val Ala His Pro Ala Ser Ser Thr Lys Val Asp Lys Lys
      85                      90                      95
Ile Val Pro Arg Asp Cys Gly Cys Lys Pro Cys Ile Cys Thr Val Pro
      100                    105                    110
Glu Val Ser Ser Val Phe Ile Phe Pro Pro Lys Pro Lys Asp Val Leu
      115                    120                    125
Thr Ile Thr Leu Thr Pro Lys Val Thr Cys Val Val Val Asp Ile Ser
      130                    135                    140
Lys Asp Asp Pro Glu Val Gln Phe Ser Trp Phe Val Asp Asp Val Glu
145      150                    155                    160
Val His Thr Ala Gln Thr Gln Pro Arg Glu Glu Gln Phe Asn Ser Thr
      165                    170                    175
Phe Arg Ser Val Ser Glu Leu Pro Ile Met His Gln Asp Trp Leu Asn
      180                    185                    190
Gly Lys Glu Phe Lys Cys Arg Val Asn Ser Ala Ala Phe Pro Ala Pro
      195                    200                    205
Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly Arg Pro Lys Ala Pro Gln
      210                    215                    220
Val Tyr Thr Ile Pro Pro Pro Lys Glu Gln Met Ala Lys Asp Lys Val
225      230                    235                    240
Ser Leu Thr Cys Met Ile Thr Asp Phe Phe Pro Glu Asp Ile Thr Val
      245                    250                    255
Glu Trp Gln Trp Asn Gly Gln Pro Ala Glu Asn Tyr Lys Asn Thr Gln
      260                    265                    270
Pro Ile Met Asp Thr Asp Gly Ser Tyr Phe Val Tyr Ser Lys Leu Asn
      275                    280                    285
Val Gln Lys Ser Asn Trp Glu Ala Gly Asn Thr Phe Thr Cys Ser Val
      290                    295                    300
Leu His Glu Gly Leu His Asn His His Thr Glu Lys Ser Leu Ser His
305      310                    315                    320
Ser Pro Gly Lys

```

<210> 72

<211> 41

<212> DNA

<213> Homo sapiens

<220>

<221> CDS

<222> (1)...(41)

<223> Human immunoglobulin light chain J-region

<300>

<308> GenBank #D90159

<309> 2002-05-29

<400> 72

```

ctc act ttc ggc gga ggg acc aag gtg gag atc aaa cgt aa
41

```

```

Leu Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg

```

1

5

10

- 137 -

<210> 73
<211> 13
<212> PRT
<213> Homo sapien

<400> 73
Leu Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg
1 5 10

<210> 74
<211> 37
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)...(37)
<223> Human immunoglobulin kappa light chain J region

<300>
<308> GenBank #D28523
<309> 2002-03-26

<400> 74
ctt tcg gcc ctg gga cca aag tgg ata tca aac gta a
37
Leu Ser Ala Leu Gly Pro Lys Trp Ile Ser Asn Val
1 5 10

<210> 75
<211> 12
<212> PRT
<213> Homo sapiens

<400> 75
Leu Ser Ala Leu Gly Pro Lys Trp Ile Ser Asn Val
1 5 10

<210> 76
<211> 37
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)...(37)
<223> Human immunoglobulin kappa light chain J-region

<300>

- 138 -

<308> GenBank #Z00020

<309> 1997-02-17

<400> 76

tgg acg ttc ggc caa ggg acc aag gtg gaa atc aaa c
37

Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
1 5 10

<210> 77

<211> 12

<212> PRT

<213> Homo sapiens

<400> 77

Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
1 5 10

<210> 78

<211> 34

<212> DNA

<213> Homo sapiens

<220>

<221> CDS

<222> (1)...(34)

<223> Human immunoglobulin light chain J segment

<300>

<308> GenBank #X72747

<309> 1994-01-28

<400> 78

gta ttc ggc gga ggg acc aag ctg acc gtc cta g
34

Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu
1 5 10

<210> 79

<211> 11

<212> PRT

<213> Homo sapiens

<400> 79

Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu
1 5 10

<210> 80

<211> 318

<212> DNA

- 139 -

<213> Homo sapiens

<220>

<221> CDS

<222> (1)...(318)

<223> Human immunoglobulin light chain Constant region

<300>

<308> GenBank #AF113889

<309> 2000-01-28

<400> 80

act gtg gct gca cca tct gtc ttc atc ttc ccg cca tct gat gag cag
48Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln
1 5 10 15ttg aaa tct gga act gcc tct gtt gtg tgc ctg ctg aat aac ttc tat
96Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr
20 25 30ccc aga gag gcc aaa gta cag tgg aag gtg gat aac gcc ctc caa tcg
144Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser
35 40 45ggt aac tcc cag gag agt gtc aca gag cag gac agc aag gac agc acc
192Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr
50 55 60tac agc ctc agc aac acc ctg acg ctg agc aaa gca gac tac gag aaa
240Tyr Ser Leu Ser Asn Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys
65 70 75 80cac aaa gtc tac gcc tgc gaa gtc acc cat cag ggc ctg agc tcg ccc
288His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro
85 90 95gtc aca aag agc ttc aac agg gga gag tgc
318Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
100 105

<210> 81

<211> 106

<212> PRT

<213> Homo sapiens

- 140 -

<400> 81
 Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln
 1 5 10 15
 Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr
 20 25 30
 Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser
 35 40 45
 Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr
 50 55 60
 Tyr Ser Leu Ser Asn Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys
 65 70 75 80
 His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro
 85 90 95
 Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
 100 105

<210> 82
 <211> 981
 <212> DNA
 <213> Homo Sapien

<220>
 <221> CDS
 <222> (1)...(326)
 <223> Immunoglobulin heavy chain constant region

<300>
 <308> GenBank #AJ250170
 <309> 2001-11-22

<400> 82
 gcc tcc acc aag ggc cca tgc gtc ttc ccc ctg gcg ccc tgc tcc agg 48
 Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg
 1 5 10 15
 agc acc tcc gag agc aca gcg gcc ctg ggc tgc ctg gtc aag gac tac 96
 Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
 20 25 30
 ttc ccc gaa ccg gtg acg gtg tgc tgg aac tca ggc gct ctg acc agc 144
 Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
 35 40 45
 ggc gtg cac acc ttc ccg gct gtc cta cag tcc tca gga ctc tac tcc 192
 Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
 50 55 60
 ctc agc agc gtg gtg acc gtg acc tcc agc aac ttc ggc acc cag acc 240
 Leu Ser Ser Val Val Thr Val Thr Ser Ser Asn Phe Gly Thr Gln Thr
 65 70 75 80

- 141 -

tac acc tgc aac gta gat cac aag ccc agc aac acc aag gtg gac aag 288
 Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys
 85 90 95

aca gtt gag cgc aaa tgt tgt gtc gag tgc cca ccg tg cccagcacca 336
 Thr Val Glu Arg Lys Cys Cys Val Glu Cys Pro Pro
 100 105

cctgtggcag gaccgtcagt ctctctcttc cccccaaaac ccaaggacac cctcatgata 396
 tcccggaccc ctgaggtcac gtgcgtggtg gtggacgtga gccacgaaga ccccgaggtc 456
 cagttcaact ggtacgtgga cggcatggag gtgcataatg ccaagacaaa gccacgggag 516
 gagcagttca acagcacgtt ccgtgtggtc agcgtcctca ccgtcgtgca ccaggactgg 576
 ctgaacggca aggagtacaa gtgcaaggtc tccaacaaag gcctcccagc ccccatcgag 636
 aaaaccatct ccaaaaccaa agggcagccc cgagaaccac aggtgtacac cctgccccca 696
 tcccgggagg agatgaccaa gaaccaggtc agcctgacct gcctgggtcaa aggtctctac 756
 cccagcgaca tcgccgtgga gtgggagagc aatgggcagc cggagaacaa ctacaagacc 816
 acacctccca tgctggactc cgacggctcc ttcttctct acagcaagct caccgtggac 876
 aagagcaggt ggcagcaggg gaacgtcttc tcatgctccg tgatgcatga ggctctgcac 936
 aaccactaca cacagaagag cctctccctg tctccgggta aatga 981

<210> 83
 <211> 108
 <212> PRT
 <213> Homo Sapien

<400> 83
 Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg
 1 5 10 15
 Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
 20 25 30
 Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
 35 40 45
 Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
 50 55 60
 Leu Ser Ser Val Val Thr Val Thr Ser Ser Asn Phe Gly Thr Gln Thr
 65 70 75 80
 Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys
 85 90 95
 Thr Val Glu Arg Lys Cys Cys Val Glu Cys Pro Pro
 100 105

<210> 84
 <211> 654
 <212> DNA
 <213> Homo Sapien

<220>
 <221> CDS
 <222> (1)...(217)
 <223> Immunoglobulin heavy chain constant region

- 142 -

<300>

<308> GenBank #AJ001564

<309> 1999-10-08

<400> 84

gca cct gag ttc ctg ggg gga cca tca gtc ttc ctg ttc ccc cca aaa 48
 Ala Pro Glu Phe Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys
 1 5 10 15

ccc aag gac act ctc atg atc tcc cgg acc cct gag gtc acg tgc gtg 96
 Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val
 20 25 30

gtg gtg gac gtg agc cag gaa gac ccc gag gtc cag ttc aac tgg tac 144
 Val Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr
 35 40 45

gtg gat ggc gtg gag gtg cat aat gcc aag aca aag ccg cgg gag gag 192
 Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu
 50 55 60

cag ttc aac agc acg tac cgt gtg g tcagcgtcct caccgtcctg 237
 Gln Phe Asn Ser Thr Tyr Arg Val
 65 70

caccaggact ggctgaacgg caaggagtac aagtgcagg tctccaacaa aggcctcccg 297
 tcctccatcg agaaaaccat ctccaaagcc aaagggcagc cccgagagcc acaggtgtac 357
 accctgcccc catcccagga ggagatgacc aagaaccagg tcagcctgac ctgcctggtc 417
 aaaggcttct accccagcga catcgccgtg gagtgggaga gcaatgggca gccggagaac 477
 aactacaaga ccacgcctcc cgtgctggac tccgacggct ccttcttctc ctacagcaag 537
 ctccacgtgg acaagagcag gtggcaggag gggaacgtct tctcatgctc cgtgatgcat 597
 gaggtcttgc acaaccacta cagcagaag agcctctccc tgtctctggg taaatga 654

<210> 85

<211> 72

<212> PRT

<213> Homo Sapien

<400> 85

Ala Pro Glu Phe Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys
 1 5 10 15

Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val
 20 25 30

Val Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr
 35 40 45

Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu
 50 55 60

Gln Phe Asn Ser Thr Tyr Arg Val
 65 70

<210> 86

- 143 -

<211> 690
 <212> DNA
 <213> Homo Sapien

<220>
 <221> CDS
 <222> (1)...(229)
 <223> Immunoglobulin heavy chain constant region

<300>
 <308> GenBank #AJ001563
 <309> 1999-10-08

<400> 86
 gag tcc aaa tat ggt ccc ccg tgc cca tca tgc cca gca cct gag ttc 48
 Glu Ser Lys Tyr Gly Pro Pro Cys Pro Ser Cys Pro Ala Pro Glu Phe
 1 5 10 15
 ctg ggg gga cca tca gtc ttc ctg ttc ccc cca aaa ccc aag gac act 96
 Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
 20 25 30
 ctc atg atc tcc cgg acc cct gag gtc acg tgc gtg gtg gtg gac gtg 144
 Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
 35 40 45
 agc cag gaa gac ccc gag gtc cag ttc aac tgg tac gtg gat ggc gtg 192
 Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val
 50 55 60
 gag gtg cat aat gcc aag aca aag ccg cgg gag gag c agttcaacag 239
 Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu
 65 70 75
 cacgtaccgt gtggtcagcg tcctcaccgt cgtgcaccag gactggctga acggcaagga 299
 gtacaagtgc aaggtctcca acaaaggcct cccgtcctcc atcgagaaaa ccatctccaa 359
 agccaaaggg cagcccgag agccacaggt gtacaccctg ccccatccc aggaggagat 419
 gaccaagaac caggtcagcc tgacctgcct ggtcaaagge ttctaccca gcgacatcgc 479
 cgtggagtgg gagagcaatg ggcagccgga gaacaactac aagaccacgc ctcccgtgct 539
 ggactccgac ggctccttct tcctctacag caggctaacc gtggacaaga gcaggtggca 599
 ggaggggaat gtcttctcat gctccgtgat gcatgaggct ctgcacaacc actacacgca 659
 gaagagcctc tcctgtctc tgggtaaatg a 690

<210> 87
 <211> 76
 <212> PRT
 <213> Homo Sapien

<400> 87
 Glu Ser Lys Tyr Gly Pro Pro Cys Pro Ser Cys Pro Ala Pro Glu Phe
 1 5 10 15
 Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr

- 144 -

```

                20                25                30
Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
                35                40                45
Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val
                50                55                60
Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu
        65                70                75

```

<210> 88
 <211> 60
 <212> DNA
 <213> Homo Sapien

<220>
 <221> CDS
 <222> (1)...(60)
 <223> Immunoglobulin heavy chain J region

<300>
 <308> GenBank #M63030
 <309> 1995-01-03

```

<400> 88
tta cta cta cta cta cta cat gga cgt ctg ggg caa agg gac cac ggt   48
Leu Leu Leu Leu Leu Leu His Gly Arg Leu Gly Gln Arg Asp His Gly
  1                5                10                15

cac cgt ctc ctc
His Arg Leu Leu
                20

```

<210> 89
 <211> 20
 <212> PRT
 <213> Homo Sapien

```

<400> 89
Leu Leu Leu Leu Leu Leu His Gly Arg Leu Gly Gln Arg Asp His Gly
  1                5                10                15
His Arg Leu Leu
                20

```

<210> 90
 <211> 60
 <212> DNA
 <213> Homo Sapien

<220>
 <221> CDS
 <222> (1)...(60)
 <223> Immunoglobulin heavy chain J region

- 145 -

<300>

<308> GenBank #M63031

<309> 1995-01-03

<400> 90

tta cta cta cta cta cgg tat gga cgt ctg ggg cca agg gac cac ggt 48

Leu Leu Leu Leu Leu Arg Tyr Gly Arg Leu Gly Pro Arg Asp His Gly

1

5

10

15

cac cgt ctc ctc

60

His Arg Leu Leu

20

ccgtctcctc

60

<210> 91

<211> 20

<212> PRT

<213> Homo Sapien

<400> 91

Leu Leu Leu Leu Leu Arg Tyr Gly Arg Leu Gly Pro Arg Asp His Gly

1

5

10

15

His Arg Leu Leu

20

<210> 92

<211> 6

<212> PRT

<213> Homo Sapien

<400> 92

Leu Leu Leu Leu Leu Arg

1

5

<210> 93

<211> 20

<212> PRT

<213> Homo Sapien

<400> 93

Leu Leu Leu Leu Leu Arg Tyr Gly Arg Leu Gly Pro Arg Asp His Gly

1

5

10

15

His Arg Leu Leu

20

<210> 94

<211> 60

- 146 -

<212> DNA
 <213> Homo Sapien

<220>
 <221> CDS
 <222> (1)...(20)

<400> 94
 tta cta cta cta cta cta ca tggacgtctg gggcaaaggg accacgggtca 50
 Leu Leu Leu Leu Leu Leu
 1 5

ccgtctctctc 60

<210> 95
 <211> 6
 <212> PRT
 <213> Homo Sapien

<400> 95
 Leu Leu Leu Leu Leu Leu
 1 5

<210> 96
 <211> 20
 <212> PRT
 <213> Homo Sapien

<400> 96
 Leu Leu Leu Leu Leu Leu His Gly Arg Leu Gly Gln Arg Asp His Gly
 1 5 10 15
 His Arg Leu Leu
 20

<210> 97
 <211> 214
 <212> PRT
 <213> Mus musculus

<400> 97
 Asp Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Val Ser Pro Gly
 1 5 10 15
 Asp Ser Val Ser Leu Ser Cys Arg Ala Ser Gln Ser Ile Ser Asn Asn
 20 25 30
 Leu His Trp Tyr Gln Gln Lys Ser His Glu Ser Pro Arg Ile Leu Ile
 35 40 45
 Lys Tyr Ala Ser Gln Ser Ile Tyr Gly Ile Pro Ser Arg Phe Ser Gly
 50 55 60
 Ser Gly Ser Gly Thr Phe Thr Leu Ile Val Asn Ser Val Gly Thr
 65 70 75 80

- 147 -

[illegible]

```
<210> 98
<211> 442
<212> PRT
<213> Mus musculus
```

<400> 98															
Glu	Val	Lys	Leu	Val	Glu	Ser	Gly	Gly	Asp	Leu	Val	Lys	Pro	Gly	Gly
1				5					10					15	
Ser	Leu	Lys	Leu	Ala	Cys	Ala	Ala	Ser	Gly	Phe	Thr	Phe	Ser	Asn	Asp
			20						25				30		
Ala	Met	Ser	Trp	Val	Arg	Gln	Thr	Pro	Glu	Lys	Arg	Leu	Glu	Trp	Val
		35					40						45		
Ala	Ser	Ile	Ser	Ser	Val	Gly	Asn	Thr	Tyr	Tyr	Pro	Asp	Ser	Val	Lys
	50					55					60				
Gly	Arg	Phe	Thr	Ile	Ser	Arg	Asp	Asn	Ala	Arg	Asn	Ile	Leu	Tyr	Leu
65				70						75				80	
Gln	Met	Ser	Ser	Val	Arg	Ser	Glu	Asp	Thr	Ala	Met	Tyr	Tyr	Cys	Ala
				85					90					95	
Arg	Gly	Tyr	Gly	Val	Ser	Pro	Trp	Phe	Ser	Tyr	Trp	Gly	Gln	Gly	Thr
			100					105					110		
Leu	Val	Thr	Val	Ser	Ser	Ala	Lys	Thr	Thr	Pro	Pro	Ser	Val	Tyr	Pro
		115					120						125		
Leu	Ala	Pro	Gly	Ser	Ala	Ala	Gln	Thr	Asn	Ser	Met	Val	Thr	Leu	Gly
		130				135					140				
Cys	Leu	Val	Lys	Gly	Tyr	Phe	Pro	Glu	Pro	Val	Thr	Val	Thr	Trp	Asn
145					150					155				160	
Ser	Gly	Ser	Leu	Ser	Ser	Gly	Val	His	Thr	Phe	Pro	Ala	Val	Leu	Gln
				165					170					175	
Ser	Asp	Leu	Tyr	Thr	Leu	Ser	Ser	Ser	Val	Thr	Val	Pro	Ser	Ser	Thr
		180						185					190		
Trp	Pro	Ser	Glu	Thr	Val	Thr	Cys	Asn	Val	Ala	His	Pro	Ala	Ser	Ser
		195					200					205			

- 148 -

```

Thr Lys Val Asp Lys Lys Ile Val Pro Arg Asp Cys Gly Cys Lys Pro
 210                215                220
Cys Ile Cys Thr Val Pro Glu Val Ser Ser Val Phe Ile Phe Pro Pro
225                230                235                240
Lys Pro Lys Asp Val Leu Thr Ile Thr Leu Thr Pro Lys Val Thr Cys
      245                250                255
Val Val Val Asp Ile Ser Lys Asp Asp Pro Glu Val Gln Phe Ser Trp
      260                265                270
Phe Val Asp Asp Val Glu Val His Thr Ala Gln Thr Gln Pro Arg Glu
      275                280                285
Glu Gln Phe Asn Ser Thr Phe Arg Ser Val Ser Glu Leu Pro Ile Met
      290                295                300
His Gln Asp Trp Leu Asn Gly Lys Glu Phe Lys Cys Arg Val Asn Ser
305                310                315                320
Ala Ala Phe Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly
      325                330                335
Arg Pro Lys Ala Pro Gln Val Tyr Thr Thr Ile Pro Pro Pro Lys Glu Gln
      340                345                350
Met Ala Lys Asp Lys Val Ser Leu Thr Cys Met Ile Thr Asp Phe Phe
      355                360                365
Pro Glu Asp Ile Thr Val Glu Trp Gln Trp Asn Gly Gln Pro Ala Glu
      370                375                380
Asn Tyr Lys Asn Thr Gln Pro Ile Met Asp Thr Asp Gly Ser Tyr Phe
385                390                395                400
Val Tyr Ser Lys Leu Asn Val Gln Lys Ser Asn Trp Glu Ala Gly Asn
      405                410                415
Thr Phe Thr Cys Ser Val Leu His Glu Gly Leu His Asn His His Thr
      420                425                430
Glu Lys Ser Leu Ser His Ser Pro Gly Lys
      435                440

```

```

<210> 99
<211> 219
<212> PRT
<213> Mus musculus

```

```

<400> 99
Asp Val Leu Met Thr Gln Thr Pro Leu Ser Leu Pro Val Ser Leu Gly
 1                5                10                15
Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Asn Ile Val His Ser
      20                25                30
Ser Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln Ser
      35                40                45
Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
      50                55                60
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
      65                70                75                80
Ser Arg Val Glu Ala Glu Asp Leu Gly Ile Tyr Tyr Cys Phe Gln Gly
      85                90                95
Ser His Val Pro Tyr Thr Phe Gly Gly Thr Lys Leu Glu Ile Lys
      100                105                110

```

- 149 -

```

Arg Ala Asp Ala Ala Pro Thr Val Ser Ile Phe Pro Pro Ser Ser Glu
      115                      120                      125
Gln Leu Thr Ser Gly Gly Ala Ser Val Val Cys Phe Leu Asn Asn Phe
      130                      135                      140
Tyr Pro Arg Asp Ile Asn Val Lys Trp Lys Ile Asp Gly Ser Glu Arg
145                      150                      155                      160
Gln Asn Gly Val Leu Asn Ser Trp Thr Asp Gln Asp Ser Lys Asp Ser
      165                      170                      175
Thr Tyr Ser Met Ser Ser Thr Leu Thr Leu Thr Lys Asp Glu Tyr Glu
      180                      185                      190
Arg His Asn Ser Tyr Thr Cys Glu Ala Thr His Lys Thr Ser Thr Ser
      195                      200                      205
Pro Ile Val Lys Ser Phe Asn Arg Asn Glu Cys
      210                      215

```

<210> 100

<211> 118

<212> PRT

<213> Mus musculus

<400> 100

```

Glu Val Met Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
  1                      5                      10                      15
Ser Leu Lys Leu Ser Cys Val Ala Ser Gly Phe Thr Phe Ser Arg Tyr
      20                      25                      30
Thr Met Ser Trp Val Arg Gln Thr Pro Ala Lys Arg Leu Glu Trp Val
      35                      40                      45
Ala Thr Ile Asn Phe Gly Asn Gly Asn Thr Tyr Tyr Pro Asp Ser Val
      50                      55                      60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Arg Asn Thr Leu Tyr
      65                      70                      75                      80
Leu Gln Met Ser Ser Leu Arg Ser Glu Asp Thr Ala Met Tyr Tyr Cys
      85                      90                      95
Thr Ser Leu Asn Trp Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr Val
      100                      105                      110
Ser Ser Ala Lys Thr Thr Pro Pro Ser Val Tyr Pro Leu Ala Pro Gly
      115                      120                      125
Ser Ala Ala Gln Thr Asn Ser Met Val Thr Leu Gly Cys Leu Val Lys
      130                      135                      140
Gly Tyr Phe Pro Glu Pro Val Thr Val Thr Trp Asn Ser Gly Ser Leu
145                      150                      155                      160
Ser Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Asp Leu Tyr
      165                      170                      175
Thr Leu Ser Ser Ser Val Thr Val Pro Ser Ser Thr Trp Pro Ser Glu
      180                      185                      190
Thr Val Thr Cys Asn Val Ala His Pro Ala Ser Ser Thr Lys Val Asp
      195                      200                      205
Lys Lys Ile Val Pro Arg Asp Cys Gly Cys Lys Pro Cys Ile Cys Thr
      210                      215                      220
Val Pro Glu Val Ser Ser Val Phe Ile Phe Pro Pro Lys Pro Lys Asp
225                      230                      235                      240

```

Val	Leu	Thr	Ile	Thr	Leu	Thr	Pro	Lys	Val	Thr	Cys	Val	Val	Val	Asp	
				245					250							255
Ile	Ser	Lys	Asp	Asp	Pro	Glu	Val	Gln	Phe	Ser	Trp	Phe	Val	Asp	Asp	
				260					265							270
Val	Glu	Val	His	Thr	Ala	Gln	Thr	Gln	Pro	Arg	Glu	Glu	Gln	Phe	Asn	
				275					280							285
Ser	Thr	Phe	Arg	Ser	Val	Ser	Glu	Leu	Pro	Ile	Met	His	Gln	Asp	Trp	
				290					295							300
Leu	Asn	Gly	Lys	Glu	Phe	Lys	Cys	Arg	Val	Asn	Ser	Ala	Ala	Phe	Pro	
305					310					315						320
Ala	Pro	Ile	Glu	Lys	Thr	Ile	Ser	Lys	Thr	Lys	Gly	Arg	Pro	Lys	Ala	
				325					330							335
Pro	Gln	Val	Tyr	Thr	Ile	Pro	Pro	Pro	Lys	Glu	Gln	Met	Ala	Lys	Asp	
				340					345							350
Lys	Val	Ser	Leu	Thr	Cys	Met	Ile	Thr	Asp	Phe	Phe	Pro	Glu	Asp	Ile	
				355					360							365
Thr	Val	Glu	Trp	Gln	Trp	Asn	Gly	Gln	Pro	Ala	Glu	Asn	Tyr	Lys	Asn	
				370					375							380
Thr	Gln	Pro	Ile	Met	Asp	Thr	Asp	Gly	Ser	Tyr	Phe	Val	Tyr	Ser	Lys	
385					390					395						400
Leu	Asn	Val	Gln	Lys	Ser	Asn	Trp	Glu	Ala	Gly	Asn	Thr	Phe	Thr	Cys	
				405					410							415
Ser	Val	Leu	His	Glu	Gly	Leu	His	Asn	His	His	Thr	Glu	Lys	Ser	Leu	
				420					425							430
Ser	His	Ser	Pro	Gly	Lys											
				435												